

Series on Testing and Assessment No. 5

REPORT OF THE SETAC/OECD WORKSHOP ON AVIAN TOXICITY TESTING

ORGANISATION FOR ECONOMIC CO-OPERATION AND DEVELOPMENT

Paris

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SERIES ON TESTING AND ASSESSMENT

No. 5

**REPORT OF THE SETAC¹/OECD WORKSHOP
ON AVIAN TOXICITY TESTING**

¹ Society of Environmental Toxicology and Chemistry

Environment Directorate

ORGANISATION FOR ECONOMIC CO-OPERATION AND DEVELOPMENT

Paris 1996

The Inter-Organization Programme for the Sound Management of Chemicals (IOMC) was established in 1995 by UNEP, ILO, FAO, WHO, UNIDO and the OECD (the Participating Organizations), following recommendations made by the 1992 UN Conference on Environment and Development to strengthen co-operation and increase international co-ordination in the field of chemical safety. The purpose of the IOMC is to promote co-ordination of the policies and activities pursued by the Participating Organizations, jointly or separately, to achieve the sound management of chemicals in relation to human health and the environment.

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FOREWORD

This document contains the report of the Workshop on Avian Toxicity Testing which took place in Pensacola, Florida, in December 1994. The Workshop was jointly organized by the Society of Environmental Toxicology and Chemistry (SETAC) and the OECD.

The Workshop report was produced within the framework of the Inter-Organization Programme for the Sound Management of Chemicals (IOMC). Derestriction was recommended by the OECD's Joint Meeting of the Chemicals Group and Management Committee of the Special Programme on the Control of Chemicals. It is published on the responsibility of the Secretary-General of the OECD.

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Executive Summary

As part of the OECD's Pesticide Programme, a Workshop on Avian Toxicity Testing was held in Pensacola, Florida, on 4-7 December 1994. It was jointly organised by the Society of Environmental Toxicology and Chemistry (SETAC) and the OECD. This was the sixteenth meeting in the SETAC 'Pellston' Workshop Series. It was chaired by Dr Peter Greig-Smith from the Ministry of Agriculture, Fisheries and Food, UK.

The specific objectives of the Workshop were to:

- (i) consider the fundamental reasons information about the toxicity of chemicals to birds is needed, and thereby identify the critical features which are essential for the tests used to generate this information;
- (ii) evaluate the positive and negative features of existing test methods, and of proposed alternatives, against the required critical features;
- (iii) develop proposals for:
 - the revision and development of OECD Test Guidelines for avian toxicity and avoidance tests, as appropriate;
 - a framework to govern the way these tests are requested and used in practice in risk assessment.

The Workshop was primarily directed towards the testing of pesticides, but it also examined the applicability of these tests to the assessment of other types of chemicals. The Workshop was organised around a series of plenary sessions and four parallel Working Groups on Acute Toxicity, Dietary Toxicity, Effects on Reproduction, and Avoidance. The discussion topics included the need for one or more tests, the use of the data generated, when the tests might be used, when they might trigger further testing, and the optimum design and flexibility of the tests.

Summary of Discussions

It was agreed by the Workshop that the use of toxicity data in risk assessments may vary, from the simple determination of a potential hazard to reducing uncertainty and producing precise estimates of risk. However, whilst addressing the wide range of toxicity data which may be required in risk assessment, the Workshop felt there was also the need to keep animal testing to a minimum but without compromising the scientific validity of the data.

The Workshop agreed that a number of features were critical to all toxicity tests which provide data for use in risk assessment. These included: applicability to a wide range of chemicals; a relevant duration of exposure; capability to provide the degree of certainty required for risk assessment; and the ability to predict effects in the environment. A number of confounding factors were identified in the existing tests, e.g. food avoidance in dietary studies, unrepresentative patterns of exposure, difficulties in extrapolating between species, and poor statistical power. These features were taken into account by the Working Groups in considering each type of test.

The Acute Toxicity Working Group recommended that three acute oral tests should be available: (1) an initial (first tier) limit test to identify toxic chemicals; (2) a definitive LD50 test (usually for a single species), normally triggered by the limit test; and (3) an approximate lethal dose (ALD) test in additional species, triggered if greater certainty is required in the risk assessment.

The Dietary Toxicity Working Group recommended a fundamental revision of the existing dietary tests to increase the exposure period from 5 days to 21 days. They recommended (1) an initial range-finding test, triggered by the LD50 and data on bioaccumulation potential, and (2) a definitive LC50 test, triggered by the range-finding test.

The Reproductive Effects Working Group recommended that: (1) the reproduction test be a first tier test (i.e. conducted on all chemicals); (2) the test duration be reduced from the current 20 weeks to 8 weeks (i.e. 2 weeks' pre-exposure and 6 weeks' exposure); and (3) the breeding performance of individual breeding pairs pre-dose be used as a covariate. The Group also identified the need to develop egg spraying, short-term exposure, and full breeding cycle tests for particular circumstances.

The Avoidance Working Group felt that, at this stage, guidance on the principles of avoidance testing was more appropriate than specific guidelines, due to the diversity of the situations in which birds may encounter chemicals. Two types of tests were recommended for chemicals classified as high-risk: (1) a critical test of avoidance under realistic conditions, and (2) an optional, more standardised screening test which could precede the critical test.

The Workshop agreed that the development of a framework for the collection of toxicity data for risk assessment could help avoid unnecessary testing. A framework was devised containing a basic (first tier) set of tests and additional tests which would be triggered only when necessary.

Summary of Recommendations for Further Work

The Workshop identified a number of areas in which work is needed for the further development of test guidelines:

Development and evaluation: (1) drafting and inter-laboratory evaluation of the recommended revisions of the dietary toxicity and reproductive effects tests; (2) submission of an ALD test guideline to the OECD; (3) development of short-term exposure and full breeding cycle reproduction tests; (4) statistical analysis to maximise the power of the proposed reproduction test; (5) further refinement and validation of avoidance testing.

Analysis of existing data: (1) identification of the appropriate numbers of individuals and species in the ALD test; (2) identification of the most efficient method of dose-response curve generation; (3) comparison of the sensitivity of juveniles and adults and of different species; (4) assessment of the reliability of laboratory to field extrapolation of avoidance.

Fundamental research: (1) field validation of reproductive effects seen in the laboratory; (2) identification of biomarkers of endocrine disruption; (3) investigation of circumstances in which avoidance fails to reduce risk; (4) investigation of the effects of sequential exposures.

Résumé

Dans le cadre du programme de l'OCDE sur les pesticides, un atelier sur les essais de toxicité pour les oiseaux s'est tenu à Pensacola en Floride, du 4 au 7 décembre 1994. Il était organisé conjointement par la Société de toxicologie et de chimie de l'environnement (Society of Environmental Toxicology and Chemistry - SETAC) et par l'OCDE. Il s'agissait de la seizième réunion de la série SETAC des ateliers 'Pellston' et elle était présidée par le Dr. Peter Greig-Smith du ministère de l'Agriculture, de la Pêche et de l'Alimentation du Royaume-Uni.

L'atelier avait pour objectifs de :

- (i) considérer les raisons fondamentales pour lesquelles des informations sur la toxicité des produits chimiques pour les oiseaux sont nécessaires, et ainsi identifier les éléments déterminants qui sont essentiels dans les essais réalisés pour générer ces informations ;
- (ii) évaluer les points positifs et négatifs des méthodes d'essais existantes et des alternatives proposées, en fonction des éléments déterminants requis ;
- (iii) développer des propositions pour :
 - la révision et le développement de Lignes directrices de l'OCDE pour les essais sur les oiseaux concernant la toxicité et la répulsion, comme approprié ;
 - un cadre pour déterminer la façon dont ces essais sont requis et utilisés en pratique lors de l'évaluation des risques.

L'atelier était principalement consacré aux essais sur les pesticides mais il s'est également intéressé à l'application de ces essais pour l'évaluation d'autres produits chimiques. Il était organisé en séries de sessions plénières et de séances de quatre groupes de travail : Toxicité aiguë, Toxicité liée au régime alimentaire, Effets sur la reproduction et Répulsion. Parmi les sujets abordés : le besoin d'un ou plusieurs essais ; l'utilisation des données produites ; quand les essais peuvent-ils être utilisés ; quand peuvent-ils déclencher la réalisation d'essais supplémentaires ; et la conception optimale et la flexibilité de l'essai.

Résumé des discussions

L'atelier a convenu que l'utilisation des données sur la toxicité dans l'évaluation des risques pouvait varier de la simple détermination d'un danger potentiel à la réduction de l'incertitude et la production d'estimations précises du risque. Cependant, tout en tenant compte du large éventail de données de toxicité pouvant être requises pour l'évaluation des risques, l'atelier a également considéré que les essais sur les animaux devaient être réalisés en nombre minimum, sans pour autant compromettre la validité scientifique des données.

L'atelier a convenu qu'il y avait un nombre d'éléments fondamentaux pour tous les essais de toxicité qui fournissent des données utilisées pour l'évaluation des risques. Ces éléments comprennent : l'applicabilité à une grande gamme de produits chimiques ; une durée d'exposition appropriée ; la capacité de fournir un degré de certitude requis pour l'évaluation des risques ; et la possibilité de prédire les effets sur l'environnement. Un nombre de facteurs qui peuvent porter à confusion ont été identifiés dans les essais existants, comme par exemple la répulsion pour la nourriture dans les études de toxicité liée au régime alimentaire, des modèles non représentatifs d'exposition, des difficultés d'extrapolation entre les espèces et la faiblesse de l'analyse statistique. Ces points ont été pris en considération par les groupes de travail pour chaque type d'essai.

Le groupe de travail sur la Toxicité aiguë a recommandé que trois essais aigus soient disponibles : (1) un essai limite initial (première séquence d'essai) pour identifier les produits chimiques toxiques, (2) un essai définitif de DL50 (habituellement sur une espèce unique), normalement déclenché par l'essai limite, et (3) un essai de dose létale approximative (approximate lethal dose - ADL) sur d'autres espèces, mis en œuvre si on a besoin d'une plus grande certitude pour l'évaluation des risques.

Le groupe de travail sur la Toxicité liée au régime alimentaire a conseillé une révision fondamentale des essais existants et l'augmentation de la période d'exposition de 5 à 21 jours. Ils ont recommandé (1) un test d'orientation initial, déclenché par la valeur de la DL50 et les données sur le potentiel de bioaccumulation, et (2) un essai définitif de CL50, déclenché par le test d'orientation.

Le groupe de travail sur les Effets sur la reproduction a recommandé que : (1) l'essai de reproduction intervienne à la première séquence (c'est-à-dire réalisé pour tous les produits chimiques) ; (2) la durée de l'essai soit réduite des 20 semaines actuelles à 8 semaines (c'est-à-dire 2 semaines de pré-exposition et 6 semaines d'exposition) ; et (3) la performance de reproduction des paires de reproducteurs avant exposition soit utilisée comme covariance. Le groupe a également identifié le besoin de mettre au point des essais de pulvérisation des oeufs, des essais pour une exposition à court-terme et sur des cycles complets de reproduction dans certaines circonstances.

Le groupe de travail sur la Répulsion a considéré que, à ce stade, des conseils sur les principes des essais de répulsion étaient plus appropriés que des lignes directrices spécifiques, en raison de la diversité des situations dans lesquelles les oiseaux peuvent être exposés à des produits chimiques. Deux sortes d'essais ont été recommandés pour les produits chimiques présentant un risque élevé : (1) un essai définitif de répulsion dans des conditions proches de la réalité, et (2) un essai de sélection, optionnel, plus standardisé, qui précéderait l'essai définitif.

Il a été convenu par l'atelier que le développement d'un cadre pour rassembler des données de toxicité pour l'évaluation des risques pourrait permettre d'éviter de faire des essais qui ne sont pas nécessaires.

Résumé des recommandations pour les travaux à venir

L'atelier a identifié un nombre de domaines dans lesquels des travaux sont nécessaires pour développer des lignes directrices pour les essais :

Développement et évaluation : (1) projet et évaluation interlaboratoire des révisions recommandées pour les essais sur la toxicité liée au régime alimentaire et sur les effets sur la reproduction ; (2) soumission à l'OCDE d'une ligne directrice ALD ; (3) développement d'essais pour une exposition à court-terme et sur un cycle complet de reproduction ; (4) analyse statistique pour maximiser la puissance de l'essai de reproduction proposé ; (5) perfectionnement et validation plus poussés des essais de répulsion.

Analyse des données existantes : (1) identification du nombre approprié d'individus et d'espèces dans les essais ALD ; (2) identification de la méthode la plus efficace pour obtenir la courbe dose-réponse ; (3) comparaison de la sensibilité entre les jeunes et les adultes et entre des espèces différentes ; (4) évaluation de la fiabilité des extrapolations du laboratoire au terrain pour la répulsion.

Recherche fondamentale : (1) validation sur le terrain des effets de reproduction observés en laboratoire ; (2) identification de bio-marqueurs du dysfonctionnement de l'endocrine ; (3) recherche des circonstances dans lesquelles la répulsion ne parvient pas à réduire le risque ; (4) recherche des effets d'expositions séquentielles.

1. Introduction

Testing the toxic effects of chemicals on birds is an established part of regulatory assessments, particularly for pesticides. National, and in some cases international guidelines for assessing acute oral, dietary and reproductive effects of chemicals on birds have been available for some years (US EPA 1982, OECD 1984a, OECD 1984b, ASTM 1990, US EPA 1993), while methods for investigating whether birds will avoid contaminated food in the environment have been developed more recently (INRA 1990, BBA 1993). These methods are now ripe for review for a number of reasons:

- Recent efforts to improve the role of ecological risk assessment in regulation have emphasised the importance of clearly defining the objectives of tests and where they fit into an overall risk assessment process.
- Many of the methods have not been revised for a number of years.
- There is a desire for international harmonization of test methods and approaches, so as to improve the quality of the information, reduce unnecessary testing, and encourage the mutual acceptance of data between countries.
- There are opportunities to address animal welfare concerns by adopting new approaches to test design, and to reduce the use of animals.
- Significant developments have taken place in the scientific understanding of animals' responses to chemicals since the tests were originally developed. These tests should now be revised to incorporate these developments.
- A substantial body of data has been obtained using existing tests, allowing an analysis of the power and consistency of these tests.

These issues were highlighted by the initiation of the OECD Pesticide Programme in 1992. As part of this programme, a survey of needs and priorities for the revision and development of OECD Test Guidelines suitable for the testing of pesticides was performed. The need for a fundamental review of avian toxicity testing was identified as being of very high priority by a task force of experts on ecotoxicology. The task force also proposed that a technical workshop would be the best way to perform this review and the best way to make specific recommendations for guideline revisions and developments.

A joint Workshop was therefore organised by the Society of Environmental Toxicology and Chemistry (SETAC) and the OECD. SETAC has a tradition of organising technical workshops involving scientists from government, academia and industry as a means of making rapid progress on issues of environmental concern, and it was felt that OECD could benefit from this experience. The Workshop was held from 4-7 December 1994 in Pensacola, Florida, USA and was chaired by Dr Peter Greig-Smith of the Ministry of Agriculture, Fisheries and Food, UK. It was the sixteenth meeting in the SETAC 'Pellston' Workshop Series. There were 48 participants from 9 OECD countries and the Commission of the European Union (see list of participants in Annex 1). Government, academia and industry were all represented.

Workshop Objectives

The Workshop's objectives were to:

- (i) consider the fundamental reasons information about the toxicity of chemicals to birds is needed, and thereby identify the critical features which are essential for the tests used to generate this information;
- (ii) evaluate the positive and negative features of existing test methods, and of proposed alternatives, against this specification;
- (iii) develop proposals for:
 - OECD Test Guidelines for avian toxicity and avoidance tests, as appropriate; and
 - a framework to govern the way these tests are requested and used in practice in risk assessment.

Focus

The focus of the Workshop was on laboratory avian toxicity tests to assess acute toxicity, dietary toxicity, reproductive effects, and avoidance of chemicals. Routes of exposure other than ingestion (e.g. dermal, inhalation) were considered, but to a lesser extent. Although directed towards the testing of pesticides, the Workshop also examined the applicability of the proposed tests to the assessment of risks of other types of chemicals.

Field tests were not addressed directly, although account was taken of the links between the results of laboratory tests and the need for tests in the field.

Background Information

Questionnaire

To help in the detailed planning of the Workshop, participants were asked to complete a questionnaire (see Annex 2) seeking their views on key aspects of the tests mentioned above and on other, more general issues. Responses to the general questions are summarised in Annex 3. Questionnaire responses in the four work areas were used as a starting point by Working Group chairs to plan each group's activities.

Comparison of existing tests

A comparison of the principal tests which currently exist or have been recently proposed for acute oral, dietary and reproductive toxicity, and for avoidance, was prepared as background information. This document is included in Annex 4.

Literature

Publications, reports and other materials which provided important background information or which were used during the Workshop are listed in Annex 5.

Workshop Structure

The Workshop was organised around a series of plenary sessions and four parallel Working Groups, which addressed Acute Toxicity, Dietary Toxicity, Reproductive Effects and Avoidance. The membership of each Working Group is given in Annex 1.

In their discussions, each Working Group was requested to consider for its particular area:

- whether one or more tests in this area would be useful for the purposes of risk assessment, and how the data would be used;
- what would trigger performance of the test(s) (i.e. when would it/they be required);
- the optimum design of the test(s) and what flexibility might be appropriate;
- whether, and under what circumstances, results from the test(s) would trigger further testing.

Report Structure

Sections 2 to 5 are the reports from the four Working Groups. They are stand-alone statements of each group's work. Section 6 develops proposals for how the various avian tests should be used in the risk assessment process (i.e. of a risk assessment framework) based on the Working Groups' recommendations. Section 7 summarises the Workshop conclusions (including views on animal welfare) and recommendations with respect to the needs for Test Guideline revision and development.

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US EPA 1993. Code of Federal Regulations.

2. Testing For Acute Toxicity

Introduction

Acute toxicity has been the classic test used to rank chemical substances for many years. In mammals, acute tests routinely include dermal, inhalation and oral routes of exposure and may include intravenous and intraperitoneal dosing. These tests have been conducted as a measure of intrinsic toxicity for human risk assessment to protect factory workers, users and the general public. In contrast, oral toxicity (LD50, LC50) is the only route of exposure for birds examined routinely for regulatory purposes, because oral exposure has been considered to be far the most important route of exposure to chemicals in the environment. Driver et al. (1991) have shown this is not necessarily always the case. However, when wildlife kills have occurred, most have resulted from oral exposure, with a few instances resulting from dermal exposure. Such incidents have involved acute toxicity following ingestion of products such as seed treatments, baits and insecticide granules containing high concentrations of pesticide. OECD currently has no acute toxicity guideline for birds, although a proposal made by Germany (1992) was submitted for consideration. Other established methods have many similarities and include US EPA 71-1 (1982) and US EPA - Code of Federal Regulations (1993). The German proposal suggest changes to current methods, particularly with respect to improved animal welfare.

Routes of Exposure

Acute toxicity testing of chemical substances in birds has been, and is still, predominantly accomplished through oral exposure using technical material or active ingredient. Until recently, the primary use of acute toxicity estimates for birds has been to rank chemicals. However, acute toxicity data have been increasingly used for risk assessment through comparison with estimated exposure in the form of a quotient. The needs for greater certainty in risk assessment, and for further improvement in regard to animal welfare issues, were the driving forces for the Acute Toxicity Working Group.

Acute toxicity testing in birds should be considered when there is any potential exposure of birds to a chemical substance. Five routes of exposure which may lead to acute toxicity can be considered: oral, oral as a result of dermal exposure, dermal, inhalation, and ocular.

Questionnaire Results

Responses to the questionnaire circulated before the Workshop (Annexes 2 and 3) confirmed the continuing need for an acute oral toxicity guideline (97%). For 'other routes of acute toxicity' it was recognised that there was inadequate guidance (96%). However, only 36% of respondents wanted a guideline for these routes of exposure at the present time.

Acute oral toxicity

The majority of respondents (65%) felt that some improvements were necessary, particularly concerning test species, numbers of birds tested, and the choice of endpoints. There was little desire to combine the acute oral test with other tests (85%). A majority

(65%) wanted the acute oral test to be used for more than just initial screening. Comparisons, especially between species, and risk assessment of products were identified as important. There was strong support for the use of an LD50, NOEL and ECx (effect threshold) as important endpoints. A majority (83%) also believed that the acute oral test could and should be used in risk assessment, but with caution.

Other routes of exposure

There were a variety of views with respect to the need for a guideline addressing other routes of exposure: 39% of respondents were in favour of *ad hoc* studies; 36% were in favour of a guideline; 18% indicated they would like a guideline in future, and 7% did not want a guideline. A small majority (57%) did not want other routes of exposure to be integrated with tests on acute oral toxicity. However, it was recognised that pen and field tests examine all routes of acute exposure simultaneously in a single study.

Purpose of the Test

Owing to the lack of knowledge concerning the importance of routes of exposure other than ingestion, the Working Group decided to summarise knowledge on 'other routes' and then concentrate on acute oral toxicity testing. The aim was to improve the risk assessment process and to recommend new guidelines and improvements to existing tests, as appropriate.

Other Routes of Exposure

It was agreed that basic work needs to be undertaken to determine the extent to which routes other than oral exposure are of toxicological relevance to birds under field conditions. The Working Group acknowledged the work by Driver (1991), which indicates that some of these exposure routes may be more important than currently imagined. The Working Group recommended that field incident reports be studied as a possible way of identifying the significance of these routes.

Possible triggers were identified which may identify the relative importance of tests addressing other routes of exposure in risk assessment, i.e.:

Triggers for inhalation studies:

- 1) vapour pressure (high volatility);
- 2) acute oral toxicity in birds;
- 3) inhalation toxicity in mammals; and
- 4) influence of formulation on exposure.

Triggers for dermal and ocular studies:

- 1) partition coefficients;
- 2) acute oral toxicity in birds;
- 3) dermal toxicity in mammals; and
- 4) the influence of formulation on exposure.

Formulation may have a greater influence on the risks from other routes of exposure than on risks due to ingestion.

The Working Group felt that there may be a need for guidance addressing other exposure routes, but agreed that at the present time there is insufficient information to develop this guidance. It was recognised that pen tests and field studies address the combined effects of all routes of exposure, but that currently it is not possible to differentiate between these routes. The Working Group recommended construction of a database on the effects of other routes of exposure, to serve as a basis for developing guidance or a specific guideline in the future.

Acute Oral Toxicity

The main use of the acute oral test in birds is in risk assessment, for comparative toxicity measurement between species (sensitivity) and for estimating variation within species under different test conditions (physiological response). Additional uses of acute oral testing in birds are labelling, setting triggers for further acute tests (other routes), and as a general guide for range-finding for other studies.

For preliminary risk assessment, a quotient approach is recommended. At least 3 quotients are either useful or currently in use:

- 1) a consumption quotient, which is a ratio calculated from the LD50 (mg/kg body weight) and estimated daily exposure (mg/kg body weight/day);
- 2) a time quotient, which is the time required (days) to consume an LD50 when feeding on contaminated food;
- 3) an area quotient in which the ratio of the LD50 in mg/bird (not mg/kg) is divided by application rate [mg/unit area].

The time quotient can be used to identify the appropriate toxicological test. When the time quotient indicates 1 day or less, the acute oral test is the appropriate test on which to base a risk assessment. Conversely, when the time quotient indicates more than 1 day, subacute dietary toxicity would be the appropriate test.

For values of the quotients that indicate a possible hazard to birds, more refined risk assessment may be necessary to reduce uncertainty. This may lead to further testing to take account of differences in sensitivity between species through exposure to formulated product, such as treated seed, bait or granules, and other factors which may have a significant influence on the risk assessment.

Initial Considerations

The Working Group felt that it was very important to define the role of the avian acute oral toxicity test, i.e. when and when not to use this test in risk assessment, and how to improve testing procedures to account for uncertainty in risk assessment, while at the same time taking into account animal welfare issues. Uncertainty and animal welfare were the key driving factors in the development of guidelines and a testing framework.

The Working Group agreed that the acute oral toxicity test is applicable to any chemical substance for which there is potential exposure to birds. It is the only test in which dietary consumption (bird behaviour) cannot influence dose. Thus it has particular utility in comparative studies. High toxicity and acute oral exposure are typical of time quotients <1 day. Thus the acute oral test is of particular relevance for chemicals applied to seeds, baits and granules used in pest management. The acute oral test cannot be used to indicate chronic toxicity and is not the test of choice in risk assessment when the time quotients are >1 day.

In acute oral toxicity testing, the most important factor contributing to uncertainty in risk assessment was seen as inter-specific differences in sensitivity to chemical substances. A higher level of uncertainty can be tolerated when the risk, i.e. using the consumption quotient, is low. Thus tests on a few species are adequate.

Animal welfare, and a recognition of the need to minimise the suffering and numbers of animals tested, were given serious consideration alongside the need for maintaining scientific integrity.

Proposal for the Acute Oral Toxicity Test

Principles of the Test

Selection of test species

There was consensus that any of the 3 conventional species (mallard, bobwhite quail or Japanese quail) could be used. If a second species is tested, it should not be another quail species. If further species are tested using the Up and Down (approximate lethal dose) method, they should come from different families to those already tested.

Current knowledge, existing databases, and the availability of mallard duck (family *Anatidae*), bobwhite and Japanese quail (family *Phasianidae*) make these the species of choice in limit and dose-response tests. Only one species per family needs to be tested. Thus a second species of *Phasianidae* should not be tested. The Up and Down method allows a wider range of species to be tested using very few animals. The choice of species used in the Up and Down method should take into consideration ecological relevance, phylogeny, and suitability for testing. The following families may provide species of ecological relevance: *Laridae*, *Columbidae*, *Corvidae*, *Turdidae*, *Sturnidae*, *Ploceidae*, *Icteridae* and *Fringillidae*. Where possible, captive bred species should be used. If this is not possible, species which are easily caught, are abundant, and acclimatise easily to test conditions should be used.

Tier placement

There was consensus that, when there is potential for birds to be exposed to a chemical substance, the acute oral test is necessary in the first tier of testing. A testing framework has been proposed which allows flexibility to meet the demands for reducing uncertainty in risk assessment and testing of animals.

The recommended series of acute oral tests is as follows:

- 1) If the chemical substance is not expected to be toxic to birds, then a single limit test only, at an upper limit dose, would be required. The limit dose is dependent on the application rate, but would not exceed 2000 mg/kg. Lower limit doses could be used where application rates are relatively low, using as a guide a dose of 2000 mg/kg for an application rate of 1 lb/acre or 1.12 kg/ha. If no toxicological effects are observed, further testing would not be required.
- 2) If a toxicological effect is observed in 1) or if a chemical is suspected to be toxic, further testing would be required to determine the LD50 and dose-response curve. A test on a single species would be required for chemicals in a well characterised chemical class. For a chemical with a new or little understood mode of action and no previously characterised dose response curve, tests on 2 species from different families would be required.
- 3) If there is uncertainty in the risk assessment, and more information on interspecies differences might be helpful to reduce this uncertainty, the Approximate Lethal Dose (ALD) should be determined on further species using an Up and Down method.

Interspecies variation, uncertainty, and the use of safety factors

There was consensus that interspecies variation in the toxicological response is a source of uncertainty in risk assessment and that the use of safety factors/uncertainty factors is necessary.¹ The Working Group recognised that, as risk increased, there is a need for greater certainty and further testing of additional species by the Up and Down method. Conversely, for a chemical demonstrating no acute toxicological response in a limit test, where the risk is low, greater uncertainty could be accepted and no further testing would be necessary. The Working Group recommended that guidance is necessary to relate numbers of species tested to safety factors.

Safety factors are necessary and are currently employed in risk assessment, but they are arbitrary. There can be quite large differences in sensitivity between species in their response to a chemical substance. The use of arbitrarily set safety factors penalises those chemicals which have been tested on a wider spectrum of species, because the same factor is applied in risk assessment to the most sensitive species tested. There have been attempts to manage uncertainty through the estimation of a threshold lethal dose which accounts for 95% of all bird species (TLD₅), (Baril et al. 1994). Baril proposed 2 options: 1) to test a battery of 6-8 species, or 2) to apply safety factors to data on a single species. The Working Group felt that the integration of both recommendations was possible. When a chemical is of low toxicity and/or exposure is low, high safety factors could be tolerated in risk assessment, thus minimising testing to a single species. Conversely, for chemicals with higher toxicity, risk assessment may demand greater certainty in both exposure and toxicity. If a refined exposure assessment does not provide adequate certainty, more species would need to be tested by the Up and Down method.

¹ Safety factors and uncertainty factors are analogous. Although 'uncertainty factor' was strongly preferred by some Working Group members as being a more objective and less value-laden term than safety factor, 'safety factor' is used throughout the Workshop report to maintain consistency between Working Group reports.

The Working Group considered that the relationship between safety factors, expressed as a consumption quotient, and the number of species which need to be tested should be investigated further. In addition, consideration should be given to recommending appropriate species for meeting criteria discussed in the section on 'Choice of species,' and to the influence of testing an additional species on the safety factors. For example, taking the data from Baril et al. (1994) and an organophosphate insecticide, what effect would using a sensitive redwing blackbird (*Icteridae*) have on uncertainty (and therefore on the safety factor) compared to the insensitive starling (*Sturnidae*) if the starling was the second species tested? The Working Group recommended that a 'White Paper' be prepared under the auspices of the OECD and SETAC by current leading researchers in this area (e.g. in Canada, Germany, the Netherlands, and the US).

Endpoints

There was consensus that mortality is the endpoint of choice, as it is the most scientifically defensible and reproducible endpoint for risk assessment and would necessitate testing of fewer animals. While the Working Group recognised the need to optimise the use of data from acute oral tests, the measurement of one endpoint must not compromise another.

A number of endpoints were identified as potentially important in risk assessment: mortality, behaviour, food and water consumption, body weight, pathological effects, regurgitation, sensory function, neurotoxicity, and physiological and biochemical responses. It was agreed that the use of data from acute oral tests should be optimised without allowing one endpoint to compromise another. For this reason, it was agreed that mortality is the single most important endpoint for risk assessment, but that other endpoints may be considered on a case-by-case basis if they are believed to be important in risk assessment. The chosen endpoints could include general measurements and those known to be specific to chemicals of similar structure or mechanisms of action. Specific endpoints may be more appropriate in higher tier tests, as they require more knowledge about the mode of action of the chemical before they can be interpreted in risk assessment.

Taking animal welfare into consideration, the Working Group felt that mortality data are scientifically robust in risk assessment and reproducible. Therefore, fewer birds would be required to establish the necessary level of certainty in risk assessment if data were based on mortality. The Working Group considered whether suffering could be reduced by using an endpoint other than mortality, similar to the approach taken in the 'Acute oral toxicity - Fixed dose method' in mammalian testing (OECD Guideline No. 420). However, the Working Group felt a similar approach in avian toxicity testing was not possible at this time. Avian toxicity testing is limited in scope compared to mammalian testing. This places additional importance on certainty in the avian acute oral test. The Working Group felt that the introduction of such uncertainty would result in more animal testing rather than less.

Description of the Methods

There was consensus concerning the limit, dose-response and Up and Down tests. The Working Group took account of existing methods and conditions from 3 guidelines, the OECD German proposal (1992), US EPA 71-1 (1982) and the US EPA Code of Federal Regulations 797.2175. The Working Group highlighted the need to examine, statistically, the most efficient way to characterise a dose-response curve using as few animals as possible. Regurgitation should be avoided, and observations improved to ensure it does not go unnoticed.

Selection of animal species

See Limit, Dose-response, and Up and Down test procedures below.

Housing and feeding conditions

These conditions must all meet the needs of the test species. Pens or cages must be of adequate size for the species being tested. The test environment (temperature and relative humidity) may be under ambient or controlled conditions. The photoperiod should be 8 hours light and 16 hours dark unless, for scientific or animal welfare reasons, a different photoperiod is necessary. Food and water should be provided *ad libitum*.

Preparation of animals

The Working Group agreed that birds should be full grown, in their first year, and not in breeding condition. It expressed a preference that the birds be reared in captivity, but agreed that wild-caught species would be acceptable. All birds must be in healthy condition and randomly assigned to control and treated groups. The minimum period of acclimation to test conditions prior to dosing must be 14 and 30 days for cage reared and wild-caught birds, respectively. Birds must be fasted for 12-15 hours overnight immediately prior to dosing.

Preparation of doses

The vehicle for gavage application is dependant on the physical/chemical properties of the test substance. Deionised water and corn oil were preferred for water soluble and insoluble substances, respectively.

Range-finding test

See Up and Down test procedure below.

Procedure

There was consensus that a selection of acute oral tests should be used to meet the requirements for precision and certainty in risk assessment. This process was considered economical in regard to the use of test animals.

Limit test

The limit test should comprise a single dose level and an untreated control. The upper limit dose should be 2000 mg/kg. Lower limit doses should be set in proportion to the application rate where 1lb ai/acre (1.12 kg ai/ha) leads to a dose of 2000 mg/kg. If any mortality or effects likely to influence survival are observed at this limit dose, a definitive LD50 (dose-response) test will be required. The limit test should comprise 1 dose level containing 6-10 birds divided into even numbers of males and females. Test animals should be young adults from any of the 3 species: mallard duck, bobwhite or Japanese quail.

Definitive LD50 (dose-response) test

The definitive LD50 (dose-response) test should comprise adequate numbers of dose levels to characterise the median lethal dose-response (LD50 and 95% confidence intervals), lowest lethal dose (LLD) and slope. If sub-lethal endpoints are being measured, the lowest observable effect level (LOEL) should also be determined. It may be necessary to run a range-finding test first. Test animals should be young adults of any of the 3 species.

The Working Group was unable to recommend the number of dose levels and birds per dose level at this time. Therefore, it recommended that a group comprising statisticians and toxicologists meet to determine the experimental design best able to provide the essential endpoints using the fewest birds. Currently 3-5 dose levels and 6-10 birds at each level is typical. A reduction in the number of animals tested may be achievable using fewer birds per dose level and more dose levels. An extreme example would be to use 1 bird per dose level and many dose levels to determine lethal endpoints and slope. If this provided the same or better precision and reproducibility, fewer than 24-60 birds might be required for these tests.

Up and Down test

The Up and Down test method may be used as a range-finding test or to evaluate the ALD for a wider spectrum of species. In the Up and Down procedure birds are dosed, 1 dose level at a time, with 2 birds per dose level. If the birds survive, the next dose level is increased; if they die the dose level is decreased. This procedure is similar to that described by Deichmann and Leblanc (1949) and Bruce (1985) and has been compared with conventional LD50 and fixed dose acute toxicity procedures (FDP) by Lipnick et al. (1995). It is convenient to allow short time intervals between dosing of individuals. This interval can be confirmed by the dose-response test. The Working Group recommended that surviving birds be monitored for delayed mortality for a total of 14 days. The test should be conducted on a single sex, if possible. If one sex is shown to be more sensitive in the dose-response test, this sex is preferred. Test animals should be young adults. Test species must be from different families and be acclimatised to test conditions without showing undue stress.

Administration of doses

The test compound should be administered, by gavage or in gelatin capsules, into the bird's crop or proventriculus. Regurgitation of the dose is a feature of acute oral toxicity testing in birds and may be related to the dosing technique or characteristics of the test substance. It must be prevented because it compromises the evaluation of toxicity. The incidence of regurgitation may be reduced by lowering dose volume or changing carriers.

Observation period

Post-treatment observation periods should last 14 days unless mortalities appear in the last 3 days. If this occurs, the observation period should be extended by 7 days.

Measurement of main endpoints

Regurgitation

Birds should be observed during the first 2 hours of the test in order to record regurgitation.

Mortality

In the absence of knowledge of the mode of action of the chemical, mortality is the endpoint of choice and is recommended by the Working Group. Mortality should be recorded during the first 2 hours of the test, at least twice during the remainder of the first day, and daily thereafter.

Behaviour

Observations of behavioural effects, including remission, should be made continually during the first 2 hours of the test and daily thereafter.

Body weight and food consumption

All birds should be weighed at least at the start and end of the study. Food consumption should be measured on days 1, 3, 7 and 14 after dosing.

Gross pathology

Gross pathology should be undertaken on all animals dying, and on up to 4 randomly selected survivors from each treatment group and controls.

Other endpoints

In special cases, when the mode of action is known and there are specific endpoints relevant to a refined risk assessment, it may be necessary to measure specific biomarkers, i.e. haematology, clinical biochemistry and histopathology.

Animal Welfare

The Working Group had a strong desire to improve animal welfare. This concern for animal welfare led to the development of a framework for using fewer animals and accepting less precision in measurement of lethal thresholds. However, the Working Group also considered that there was a significant need for greater certainty in risk assessment to protect wild species. The Working Group considered that this need would be satisfied by minimising the testing of low risk chemicals and testing more species using the Up and Down method when risk was more uncertain. The Working Group was reluctant to accept any endpoint other than mortality, because of difficulties with interpretation of sub-lethal endpoints and the likelihood that such endpoints would lead to the testing of more animals. Its recommendations to identify objectively the numbers of species and minimum number of test animals necessary is evidence of a commitment to animal welfare.

Recommendations for Further Work

1. A 'white paper' should be prepared to identify the number of species and the utility of ALD's (Approximate Lethal Dose) required in testing to provide adequate certainty in risk assessment or, in the absence of testing of additional species, the appropriate uncertainty factors. This paper should also recommend the appropriate test species. Research to date in this area has been carried out in Canada, Germany, the Netherlands and the US. A group, including representatives of these countries, should participate in producing this paper.
2. Additional test species, different from those tested in the limit and dose-response tests, may be used in the Up and Down test. These species should come from different families, and consideration should be given to the practicality of testing different species within these groups.
3. The Avian Up and Down test procedure outlined above should be submitted to the National Co-ordinators of the OECD Test Guidelines Programme.
4. The National Co-ordinators of the OECD Test Guidelines Programme should evaluate the most efficient way to generate a dose-response curve, with special consideration given to the number of animals per dose level and the number of dose levels.

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3. Testing For Dietary Toxicity

Introduction

This section presents the discussions of the Dietary Toxicity Working Group.

Data from dietary toxicity tests have been used for many years in risk assessment. A number of test guidelines have been produced, e.g. OECD Test Guideline 205, US EPA 71-2, US EPA Code of Federal Reg. 797.2050. However, the Working Group perceived a number of drawbacks in the design of the current dietary (LC50) toxicity tests. These problems included:

- Food avoidance is a confounding factor in determining the lethality of a compound in feed.
- The lack of replication in current protocols limits the power of the test.
- The test design prevents good characterisation of sub-lethal effects.
- The duration of the test does not accurately reflect the patterns of exposure which occur in the wild.

The group addressed these problems through consideration of the objectives and design of a dietary toxicity test, whilst taking into account the use of the results for risk assessment or other purposes.

Purpose of the Test

The Working Group agreed that there is a need for a dietary toxicity test that provides the dose-response relationship from dietary exposures lasting from a few hours to 3-4 weeks. This exposure period is not covered by the single dose acute oral toxicity test or the chronic reproduction test.

The Working Group also agreed that the test should not be designed to simulate realistic field conditions, but that it does need to be relevant to field exposures.

The results of the dietary toxicity test may be used for risk assessment or to trigger additional testing as follows:

- If the test provides an LC50, it may be used to carry out risk assessment.
- Food consumption data may also help explain what is happening in the test and allow an estimate to be made of threshold concentrations at which deaths will occur.
- Other endpoints allow the evaluation of how the chemical might impact the fitness of the animal in the environment, or can be used to interpret the results of field tests, field monitoring, or wildlife forensics.

- If decreased food consumption that can be attributed to food avoidance behaviour is observed, an avoidance test may be considered.

It should be noted that the Working Group was not confident that reduced food consumption should trigger an avoidance study without further evidence of food avoidance. Most tests show some reduced food consumption, but this is most likely to be due to toxicosis rather than avoidance. Compensatory eating during the recovery period could be an indication of avoidance or toxicosis.

Initial Considerations

In considering the dietary toxicity test, the following issues were discussed:

- Should the test characterise dietary toxicity due to 'sub-chronic' exposure?
- Should the test determine effects of field dose rates?
- Should the test reflect persistence in the field?
- Should the test provide a dose-response curve?
- Should the test provide secondary endpoints such as palatability?
- Should it be a test with juveniles, sub-adults or adults?
- Should it be a second tier test, i.e. LD50 test always carried out first?
- Should it be useful for classification of acute toxicity, LD50 versus LC50?
- Should the test give an indication of cumulative toxicity?
- Should this test be used as a trigger for avoidance testing?
- Should this test be used as a replacement of the LD50 test: more realistic measure of exposure ranging from hours to several weeks?
- How should food avoidance be dealt with?
- How much would we like to achieve in one test, and to what extent is this tier-dependent?

There should be no substantial differences in the design of dietary tests for chemicals that are not pesticides.

Proposal for the Dietary Toxicity Test

The following sections record the proposed dietary toxicity test design and the discussions leading to these conclusions.

Principles of the Test

The overall principle of the test is to administer the test substance, via the diet, at graduated concentrations (determined in a range-finding test) to several groups of experimental birds, one group per concentration level, for a period of 21 days. During the period of exposure the birds should be observed closely each day for signs of toxicity. Birds which die or are killed during the test should be necropsied. At least a sample of the surviving birds from the top dose should be necropsied and studied for evidence of toxic or pathological changes at the end of the treatment. The other surviving birds should be fed an untreated diet for 7 additional days to assess recovery. At the end of the recovery period all surviving birds should be killed and necropsied.

Selection of test species

The Working Group recommended that the preferred test species should be bobwhite quail or mallard.

It was generally agreed that inherent differences in sensitivity between species could be better assessed in LD50 studies than in dietary studies. Therefore, dietary testing of multiple species is not necessary if LD50 tests on multiple species have already been conducted. If two or more LD50 values were available, then only a single dietary study is needed. This test would be performed with the most sensitive species in the LD50 tests that was also amenable to dietary testing. If only one LD50 value is available, dietary studies of two species would be needed to give some indication of interspecies differences in sensitivity.

The preferred test species are the bobwhite quail or mallard, although ring-necked pheasant and red-legged partridge are also potential test species. Japanese quail are not recommended, as birds should not be in reproductive condition during the test and this is difficult to achieve in that species. The group considered the use of a passerine species as a possible test species in cases where testing of multiple species was desirable, but some species may not be amenable to laboratory testing.

Tier placement

There was consensus that the dietary toxicity test should usually be a second tier study dependent on the results of the acute oral toxicity test.

Agreement was reached within the group that the dietary test is not necessarily needed for all compounds. The need for dietary toxicity tests can be summarised as:

- 1) Are all available LD50s³ \geq 2000 mg/kg body weight² and is the Kow < 1000³ and do mammalian studies show no evidence of bioaccumulation?

² For pesticides applied at rates <1.12 kg/ha, lower limits could be used (see Section 2, Tier placement).

³ The group recognised that Kow is not the most appropriate parameter to indicate possible bioaccumulation in birds, but that it may be used in a precautionary way.

if yes -----> No definitive dietary toxicity test is necessary. Stop.
if no -----> Conduct a range-finding dietary toxicity test and go to 2.

2) Is the LC50 from a range-finding dietary toxicity test \geq 5000 mg/kg food?

if yes -----> No definitive dietary toxicity test is necessary. Stop.
if no -----> Go to 3.

3) Are the results of more than one LD50 test available?

if yes -----> Carry out dietary toxicity test for the most sensitive species (based on LD50).

if no -----> Carry out dietary toxicity test for two species: 1) bobwhite quail, ring-necked pheasant or red-legged partridge; and 2) mallard.

Methods of dosing

The Working Group recommended that only dietary exposure should be used.

The group discussed the relative advantages and disadvantages of multiple oral doses as a substitute for dietary exposure in order to reduce food avoidance effects:

Dietary exposure

Advantages

- more realistic exposure
- could indicate avoidance effect
- limited regurgitation
- no handling stress

Disadvantages

- food avoidance may be a confounding factor
- amount ingested by each bird cannot be controlled
- precise measurement of amount ingested is impractical

Multiple oral dosing

Advantages

- enables precise measurement of dose
- food avoidance is due only to toxicity
- useful for volatile or unstable compounds

Disadvantages

- daily dose may kill at levels not lethal in dietary test
- regurgitation may occur
- starvation may still be a confounding factor
- increased stress from handling birds
- lacks realism

The Working Group agreed that because the objective of the test is to measure dietary toxicity, dietary exposure is the most realistic. In addition, it was acknowledged that the daily dose administered via a multiple dose study can cause mortality at levels not lethal in dietary toxicity studies.⁴

⁴ During the review of this report, it was pointed out that multiple oral dosing may be necessary for very volatile or unstable compounds.

Test duration

The Working Group recommended that the test duration should be 21 days, followed by a recovery period of 7 days.

The group discussed whether the current 5-day test duration was adequate. It was agreed that dietary exposure in the field ranges from a single day to several weeks. A 5-day test therefore provides only some of the necessary information. From a risk assessment standpoint, it may be of interest to describe the time-course of mortality and compare the duration of expected exposure in the field with the duration of exposure necessary to cause effects. It was also agreed that a powerful advantage of a longer test period is the ability to characterise effects over any shorter timespan, whereas the reverse is not true. If field exposure was limited to 7 days, only the effects occurring in the first 7 days of the dietary toxicity test would be relevant in characterising the effects for field use.

The consensus was that both mortality and other, sub-lethal endpoints should be measured for 21 days, followed by a 7-day recovery period. Dissent was registered to this approach since a minority felt that mortality should be measured over a shorter time period when less persistent, non-bioaccumulating compounds were being tested.

Description of the Methods

Age of test birds

There was consensus that birds should be 42±2 days at the start of the acclimation period.

The group debated the relative merits of using juvenile birds or young adults:

Juvenile birds

- | | |
|---------------|---|
| Advantages | <ul style="list-style-type: none">- lower costs- existence of historical database |
| Disadvantages | <ul style="list-style-type: none">- gang housing confounds information- food avoidance may cause mortality- low replication for the number of individuals used- higher probability of background mortality from other causes |

Young adults

- | | |
|---------------|--|
| Advantages | <ul style="list-style-type: none">- individual housing allows precise measurement of endpoints- less susceptible to death from starvation- able to estimate amount of chemical ingested per bird- enables measurement of additional endpoints, e.g. haematology |
| Disadvantages | <ul style="list-style-type: none">- higher costs |

From the above it was agreed that young adult birds should be used. There was some discussion as to whether juvenile birds were more sensitive than young adult birds. It was felt by some members of the group that any differences might be due to the greater

susceptibility of juveniles to death by starvation, rather than to differences in the toxicity of the compound. Therefore, the group recommended that the comparative sensitivity of the two age groups to the toxic effect of chemicals should be reviewed to ensure the recommended approach was correct.

It was agreed that birds should be old enough to be housed separately, but young enough not to reach breeding condition during the test. This ruled out the use of Japanese quail, since they would reach sexual maturity during the test. Dissent was registered on this point (i.e. a light regime of 8 hours light and 16 hours dark and should prevent Japanese quail from reaching breeding conditions). The consensus was that birds should be 42 ± 2 days at the start of the acclimatisation period.

Housing and feeding conditions

The Working Group agreed that birds should be individually housed, with the size of cage, temperature and humidity conditions appropriate for the species and age. A standard test diet should be used, and the caloric content should be determined.

Birds should be individually housed, since this enables food consumption to be measured easily. Individual housing increases replication and, as a result, the power of the test. However, the group discussed with a statistician whether any power was really gained by individually housing birds. An LC50 value could not be calculated using the measured dose via food consumed, as each bird within a test concentration would ingest a different dose and the dose would result in either 0% or 100% mortality. However, partial mortality information to calculate an LC50 value could be obtained by considering multiple cages (birds) within a concentration level.

The size of test cage should be appropriate for the species and age of the bird. Standard temperature and humidity conditions appropriate for the test species should be used. An 8-hour light: 16-hour dark photoperiod was recommended.

Preparation of animals

The Working Group agreed that a 7-day acclimatisation period should precede the 21-day test period.

Preparation of doses

There was a consensus that at least 5 test groups and a control group should be used, with the highest dose selected to result in approximately 85% mortality.

At least 5 test groups and a control group should be used. However, if exposure periods of more than 10 days could be expected under use conditions, additional test groups should be considered. Animals in the control group should be handled in an identical manner to the test group subjects.

Diets should be mixed and concentrations verified following standard procedures described in guidelines for avian dietary studies. The test should be performed using the active ingredient, rather than a formulation, unless information from other tests (e.g. mammalian tests) indicates that a formulation may be significantly more toxic.

Dose levels should be selected taking into account any existing toxicity and (toxico-) kinetic data available for the test compound or related materials. Data from the range-finding test will be available because this test preceded the dietary toxicity test. The highest dose level should be chosen to cause 85% mortality. Thereafter, a descending sequence of dose levels should be selected with a view to demonstrating any dose-related response.

Range-finding dietary toxicity test

If a range-finding dietary toxicity test shows that the LC50 is greater than 5000 mg/kg diet, a dietary toxicity test is not necessary.

Procedure

Number and sex of test birds

The Working Group agreed that the number of test birds should be based on the minimum number necessary to obtain an adequate dose-response characterisation for the primary endpoints of the study (mortality, body weight and food consumption).

The group discussed whether characterisation of the dose-response curve could be improved by increasing the sample size in the highest and lowest doses, or by increasing the number of birds in the second-highest and second-lowest dose levels, because these levels might correspond more closely to the LC84 and LC16 levels. Following advice from a statistician, the group proposed that a greater number of birds should be placed in the highest and lowest dose levels.

The question of the number of birds per test concentration was revisited. It was agreed that sex differences in toxicity could be better evaluated in an LD50 study, and that unless such studies showed a difference between sexes, it was not necessary to have as many birds in the dietary toxicity test. The following numbers per test level were recommended, with the understanding that each test level would contain equal numbers of males and females:

Control	14
T1 (lowest)	16
T2	10
T3	10
T4	10
T5 (highest)	16

The above numbers may need to be increased in cases where sex-related differences in toxicity have been demonstrated.

Administration of the doses

Birds should receive the test compound via their diet for a 21-day period or until death (or a decision that death is imminent when the bird is sacrificed).

In tests using mallard ducks, it was recommended that water should be supplied in sipping tubes to prevent messing of the diet.

Recovery period

The Working Group recommended a 7-day recovery period at the end of the test, to demonstrate whether any gross pathology, histopathology or biochemical endpoints were reversible.

It was agreed that the recovery period could be extended if mortalities occur during the recovery period. It should be noted that birds may take more than 7 days for recovery to normal from some debilitating but non-lethal pathological effects.

Measurements of main endpoints

It was agreed by the Working Group that endpoints need to be quantifiable, interpretable and significant. It was further agreed that the primary study endpoints (i.e. the endpoints for which the study is designed to make a statistical evaluation) are mortality, body weight and food consumption.

Mortality

The group recognised that there is a conflict between the characterisation of lethal and sub-lethal effects. These endpoints require different dose ranges. Refinement of the dose-response curve also conflicts with the refinement of sub-lethal parameter effects. It was agreed that the main endpoint of the dietary toxicity test should be mortality.

Further discussions included whether the endpoint for mortality could be the threshold level at which deaths occurred rather than the calculation of the LC50 from a dose-response curve. Problems were encountered in regard to the statistical relevance of the threshold approach. Therefore, it was agreed that a reliable measure of mortality, including the threshold level, could only be achieved by a well characterised dose-response curve.

Food consumption

Food consumption should be measured daily throughout the acclimatisation period, test period and recovery period, with the exception of the first day of the test period and the first day of the recovery period, when it should be measured twice.

Body weight

Measurements of body weight should be taken once a week from the beginning of the acclimatisation period until the end of the recovery period. It was recognised that water retention is a confounding factor which may affect body weight measurements.

Clinical signs

Although clinical signs are neither quantifiable nor objective, the group agreed that they provide useful additional information and should be recorded. Faecal consistency should be included as a clinical sign. Observations should be made twice daily, from the beginning of the acclimatisation period to the end of the recovery period. It was recommended that detailed behavioural observations should not be included.

Clinical chemistry

It was acknowledged that interpretation of biochemical endpoints is problematic. However, the group considered that they provide useful additional information which could indicate effects in conjunction with other endpoints (see Appendix to this section). Concern should be for irreversible effects, i.e. if no signs of recovery are displayed during the recovery period at the end of the test.

If measurements of clinical chemistry are made, they should be taken during the acclimatisation period, and on day 21 for at least 5 birds per dose level. Birds should be randomly selected and act as their own control.

Gross pathology and histopathology

Gross pathology and histopathology should be measured to give an indication of health of birds at sub-lethal doses and in the control. Concern was expressed about the ability to interpret measures of gross pathology and histopathology, including biochemical markers. However the consensus was that these measures provide useful additional information in combination with other indicators of health at sub-lethal doses (see Appendix). The group was concerned about the additional costs associated with measuring these endpoints, as well as their interpretability and usefulness, and dissent was registered. It was agreed that the targets for gross pathology and histopathology measurements should be the liver, kidney, spleen, thyroid and gonads for all birds that die during the test. The presence/absence of subcutaneous fat should also be recorded for dead birds.

At 21 days, half of all surviving birds from each group should be sacrificed and brain, kidney, liver, spleen and gonad weights determined. In addition, histopathological observations should be conducted at the end of the test on the liver, kidney, spleen, gonads and thyroid for both control birds and birds at the dose exhibiting approximately 50% mortality.

Histopathology should also be undertaken at the end of the recovery period for any birds which exhibit adverse effects during the test.

Animal Welfare

Concern was raised as to whether killing test birds could be justified, i.e. is a dietary toxicity test really necessary in addition to the acute oral toxicity test? The group agreed that both an acute and a sub-chronic toxicity test are needed, since they reveal effects resulting from different exposures. The dietary test also provides additional information necessary for an adequate risk assessment, and the need for qualitative information should not be compromised by animal welfare concerns. Whether fewer birds could be used at individual dose levels should, however, be explored further.

There was agreement that tests producing prolonged reductions in food intake, to the point where birds could starve, should be stopped when this occurrence is noted.

One of the reasons for obtaining information on endpoints other than mortality, body weight and food consumption in the dietary toxicity test is the consideration that the birds are already being used in a toxicity test. Measuring other endpoints may avoid the need for additional testing.

Recommendations for Further Work

1. A comparative review of the sensitivity of juvenile and sub-adult birds to the toxic effect of chemicals should be conducted.
2. Research is needed to evaluate the proposed test design using a 21-day exposure period of sub-adult animals. Further consideration should be given to whether fewer birds could be used.
3. If successfully developed, the new test design should be subjected to inter-laboratory testing (ring-testing) prior to adoption as a standard guideline.

Appendix: Uses and interpretation of haematology, clinical chemistry, and histopathology/organ weight information

The sub-chronic dietary toxicity test will provide information to the risk assessor about mortalities associated with consumption of diet containing the test material, as well as additional information about the health of animals at sub-lethal exposure concentrations. Animal health affects the fitness of individuals, which, in turn, can reduce survival or reproductive capabilities over the long term. Sub-lethal health effects of chemical exposure have been shown to interact with environmental stressors such as adverse climatic conditions, food or water stress, and disease agents to reduce survival and reproductive success in animals (e.g. deer mice, *Peromyscus maniculatus*; Porter et al. 1984). Therefore, they are important and must be considered in evaluating the acceptability of environmental contaminants.

The sub-chronic dietary test procedure includes selected measurements of haematology, serum chemistry, organ weights, and gross and microscopic histopathology. These endpoints, when evaluated in a weight-of-evidence approach, provide valuable information about overall animal fitness and provide the risk assessor with insight into possible adverse effects that may result from longer-term exposures. Briefly, the following measures are recommended for inclusion in the test:

- Haematological or blood measures considered in the study design include red and white cell counts, differential counts, haemoglobin, and packed cell volume. This information provides insight regarding a chemical's ability to cause anaemia (i.e. a reduction in red cell parameters, which might impair an animal's ability to run or otherwise exert itself) or whether it can alter the number and type of white cells, resulting in impaired ability to fight off pathogens or parasites.
- Clinical chemistry measurements include several minerals and electrolytes (e.g. calcium, phosphorous, chloride) as well as several metabolic products and enzymes (e.g. blood urea nitrogen, alanine aminotransferase) that provide a non-destructive means for evaluating the general condition of various organs, tissues, and biochemical or metabolic systems. The test procedure includes a measurement of function for each of the major organs (heart, liver, kidney) plus cholinesterase. A failure or impaired function of a major organ will reduce an animal's ability to metabolize food, excrete waste products, circulate blood, and maintain general homeostatic processes. Calcium and phosphorus measurements will provide additional information on potential reproductive effects, as birds are very dependent upon proper calcium metabolism for proper egg shell production.
- In many cases, chemical exposure can increase or decrease organ weights. When normalized on the basis of body or brain weight, organ weights can provide an indication of malfunction or compensatory responses that can arise as a result of chemical exposure. Organs to be collected and weighed include liver, spleen, kidney, gonads, thyroid and brain.

- Gross and microscopic histopathological examination of organ tissues can provide a description of the mechanism(s) causing changes in organ weight or biochemical parameters through evaluation of structural changes. It provides an additional description of the severity of the effect. (See Fairbrother, 1993 for a detailed discussion of the methods and interpretations for wildlife clinical biochemistry.)

Evaluation of these parameters will provide the risk assessor with a quantitative basis for evaluating animal health. However, the interpretation of the biological significance of any single finding must be considered in light of all of the available data. The finding of a statistically significant difference (either as an increase or decrease) in a single clinical parameter would mean little unless accompanied by supporting data suggesting an adverse change in the structure or function of an organ, tissue, or biochemical pathway. Changes in these types of parameters may be indicative of either a chemical-induced adverse effect on the fitness of that individual or a compensatory response (i.e. a change in structure or function as a result of metabolic response processes). Interpretation of the biological significance of any finding must consider its ability to compromise the survival or reproductive potential of an animal. In general, a conclusion of biological significance must be based on three factors. Changes must:

- occur in a dose-response fashion (i.e. more abundant or pronounced in higher exposure groups);

Note: Occasionally, toxic responses do not follow simple dose-response curves (e.g. depletion of biochemical material involved in the detoxification mechanism can produce step functions in toxicity).

- be accompanied by confirmatory changes (i.e. differences in a biochemical parameter or organ weight, or histologically observable changes in tissue structure); and, most importantly,
- be related to an adverse condition that would compromise the ability of the animal to survive, grow or reproduce in the wild.

For example, a statistically significant change in all haematological and serum chemistry parameters between chemical-exposed and control animals would indicate sufficient concern to warrant restriction of the chemical at that concentration. Additional lower concern levels would be triggered by changes in all red cell parameters (number, haemoglobin, and packed cell volume) or in white cell types and numbers. A significant change in any one of the organ chemistry measures, coupled with a histopathological change, should also trigger a risk assessment decision, as a fitness effect would be likely to occur. A change in only a single parameter should raise concern and a discussion of possible additional studies, but there would probably not be enough information upon which to base a risk assessment decision.

In summary, because animals must be exposed to toxicants in the assessment process, we should strive to obtain the most information possible. For a relatively small investment of time and money (compared with the overall cost of the test), a great deal of information can be obtained to allow the risk assessor to evaluate how the chemical might impact the fitness of the animal in the environment. Some of the suggested data endpoints do not have a great deal of historical precedent in the field of avian toxicology; however, with experience they may become integral to the toxicity evaluation process, just as they

have in mammalian toxicology. By combining sub-lethal endpoint data with additional information from the reproduction test and measures of lethality, the uncertainty of the hazard estimate can be reduced, thereby enhancing the predictive ability of the risk assessment as a whole. Finally, information gained during this pre-registration testing process can be directly applied to field diagnostics, either in field tests, field monitoring, or wildlife forensics.

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4. Testing for Effects on Reproduction

Introduction

It has long been recognised that chemical effects on reproduction are potentially of the highest ecological relevance, and that the detection of such effects should be a high priority for regulatory bodies. There is ample historical justification for this concern. Much of the impact of the organochlorine insecticides, DDT especially, was mediated through a decrease in reproductive function.

A test for reproductive effects in birds is currently part of the regulatory ecotoxicology requirements for pesticides in many countries. Of the three tests currently mandated in birds (the LD50 and dietary LC50 tests being the others), the reproduction test is the most time-consuming and most expensive. This test is the only standardized one which focuses on toxicity endpoints other than death and which requires sub-chronic dosing of the test individuals. The EPA protocol for the avian reproduction test has been the "industry standard" for such tests. It was first introduced in 1975 and further refined in 1978 and 1982 (US EPA 1982, McLane 1986). Essentially the same protocol was subsequently adopted by the Organisation for Economic Co-operation and Development (OECD, 1984) and recommended by the American Society for Testing and Materials (ASTM, 1990).

Over the years, concerns have been expressed over the continuing relevance of this test. It was designed principally to detect eggshell thinning and other impacts resulting from the bioaccumulation of chlorinated hydrocarbon insecticides. Pesticides are therefore administered to the birds over a lengthy period to allow for bioaccumulation to occur, an exposure profile which does not correspond to the persistence characteristics of most modern pesticides. In addition, the two species mandated by the US and therefore most often tested, the mallard and bobwhite quail, are acknowledged to have been chosen in large measure because they are important American game species rather than for any toxicological attribute or for the ease with which testing could be carried out. However, the availability of these species and the fact that they reliably breed in captivity were obviously important considerations. The usefulness of the avian reproduction test, as currently designed, has been debated in regulatory circles. Future modifications of the test in the United States and elsewhere have been proposed in recent years (Bennett and Ganio 1991, Dobson 1992, Mineau et al. 1994). A proposal for a totally redesigned test using the Japanese quail as test species was formally submitted by Germany for consideration by the OECD in March 1993 (OECD 1994a).

Questionnaire Results

Results of the questionnaire circulated to Workshop participants confirmed that most were dissatisfied with current guidelines (see Annex 3). Major areas of dissatisfaction were length of the study, test species, and endpoints. Views tended to be very polarized, and statements from solicited experts were very often at odds. What follows is a very brief summary of the 30+ responses received.

Length of study

There was the least amount of disagreement between respondents over the length of the test. Only one respondent commented favourably on current length of testing, while most thought the test too long but for differing reasons. Some emphasized the short-lived nature of most current pesticides, whereas others highlighted the disadvantages of the long dosing and egg-laying periods, namely loss of statistical power and inability to provide exposure levels high enough to be realistic. A number of respondents had a specific test length to recommend, ranging from about 4 to 10 weeks.

Test species

There were clear differences of opinion on the number of species which should be tested and on the identity of those species, but most respondents were in favour of greater flexibility. Several respondents (including those who endorsed the German draft protocol) wished the Japanese quail to be recognised more widely as a valid test species. This species is allowed in the current OECD test protocol, but not recognized by the US. On the other hand, some respondents felt there were problems specific to the Japanese quail, at least in the context of current protocols, because it is difficult to maintain the birds in a pre-laying state. Some respondents were also concerned that the relative sensitivity of the Japanese and bobwhite quail was not known.

Endpoints

On the question of endpoints, the differences in responses were striking. On one hand were those respondents who wanted to limit the endpoints to those currently measured or reduce them further. On the other were many who would have liked to see the net broadened to include a suite of behavioural, biochemical and histological endpoints so that more information could be 'squeezed' from the studies. A few respondents argued that inclusion of new endpoints might simplify the test, or at least make the interpretation easier. Finally, the idea of a 'tiered' reproduction study was proposed, with some of the biochemical and histological endpoints relegated to higher tiers.

Realism

A number of respondents were critical of the current lack of realism in the test protocols (e.g. eggs are removed when laid and artificially incubated), while others indicated that realism was not the intent of the test and that there were good reasons not to make the test too realistic, for example by allowing natural incubation.

Statistical power

Most respondents commented that the statistical power of the current test was insufficient. Several suggestions were made such as the use of proven breeders, increased sample sizes, and new endpoints, as well as new and standardized ways to analyse the data.

Second generation studies

The majority of respondents did not believe it was necessary to extend the reproduction study to a second generation, although several commented on the need to screen for endocrine disrupters.

Dose levels

On the subject of dosing and dose levels, two alternative philosophies were apparent; some respondents believed doses should be set from label rates so as to be 'realistic' or 'meaningful' and others advocated setting dose levels purely on toxicological grounds, with the idea that the test is not realistic and that the use of a 'realistic' dosing level ignores interspecies variation.

Role of a reproduction study

Perhaps most informative were the responses to a question which tried to address the role or value of an avian reproduction study. **Table 4.1** shows the primary reasons for conducting an avian reproductive test, which respondents were asked to rank in order of their relative importance.

Table 4.1 Primary reasons for conducting an avian toxicity test

Reasons:	Mean rank ¹	Judged most important	Judged least important ²
To simulate avian reproduction under realistic conditions of exposure.	2.9	4/30	14/30
To show a substance's potential to affect the reproductive system of birds.	1.9	15/30	5/30
To act as a possible trigger for field studies and help in their design.	3.0	1/30	13/30
To simulate a chronic exposure situation, and define a maximum tolerated concentration for birds.	2.2	10/30	6/30

¹ Where 1 is ranked most important, and 4 is the least important.

² Totals will not add up to total number of respondents (30) because of tied ranks.

The lack of a clear consensus on the role of the avian reproduction study was apparent, especially the fact that each reason was judged the most important by at least one respondent. This indicated the need for the Workshop to revisit some of the basic premises on which the reproduction study is based.

The following sections represent the consensus position of the Avian Reproduction Working Group. Consensus statements are highlighted at the beginning of each sub-section. They reflect the proceedings of the Working Group deliberations, and are followed by a discussion of the arguments behind each statement.

Purpose of the Test

The overall objective of the test is to evaluate the effect of a test chemical on avian reproductive performance and to provide a mechanism, when the data are combined with exposure information, for predicting effects on wild birds. To be ecologically meaningful,

predictions stemming from this test should relate to effects at the population level. However, legal and social considerations often require that individual-level effects be ascertained, even if these effects are ultimately considered to be acceptable in view of a cost/benefit argument. Furthermore, whilst the ideal of the reproduction test is to reach conclusions at the population level, this is seldom, if ever, achievable in practice. One reason is that it is very difficult to project impacts seen on individuals in the laboratory to individuals, let alone populations, in the field. Moreover, several species may be affected, including locally rare or vulnerable species or species less able to compensate for a loss of production ability.

The reproduction test aims to monitor reproductive parameters during a relatively long-term (sub-chronic) chemical exposure of selected test species. Production of sexually viable chicks (by which is meant chicks that have the potential to mature into sexually viable adults) is the most important endpoint, though other intermediate endpoints are also measured. These include, but are not restricted to, onset of lay, numbers of eggs produced, eggshell parameters, fertility and hatchability. Intermediate endpoints give information on the mechanisms of toxicity which contribute to the overall breeding success. It is critical that all endpoints be taken into account when using the results from the test for risk assessment. The ecological significance of effects on each of the parameters measured may differ.

The test represents a simple model of gonadal functionality in birds exposed to the chemical of concern. The test was never meant to accurately reflect a bird's full breeding cycle. Therefore, it cannot be overemphasized that the test is not 'realistic' for avian breeding efforts in the wild and that this lack of realism needs to be considered in the risk assessment.

The statistical power of the test should be a major consideration in the design of the test protocol. Tests should be statistically robust and scientifically defensible. However, given the many intermediate endpoints prior to the measure of sexually viable chicks produced, the numbers of animals needed to produce adequate power for all endpoints may be unrealistically high. Given that the overall production of sexually viable chicks is the main endpoint (see above), test power should be adequate to show an effect in this endpoint at least.

The potential for iterative investigation of reproductive effects is currently very limited. The reproduction test represents a single opportunity to observe reproductive endpoints without flexibility in approach. The possibility of varying the protocol in the course of a study to take account of intermediate observations seems attractive. However, this entails the risk of compromising the power or rigour of the test methods and was considered by the group to be undesirable at present or difficult to achieve under GLP testing. Therefore, the consensus was that the test protocol should be fixed and that flexibility should come at higher tiers of testing, with custom-designed tests to explain reproductive effects seen in the original study.

Initial Considerations

The consensus was that a basic test of reproduction in birds (described below) was suitable for all chemicals. The test should be used for all pesticides, and should also be used to test all major use chemicals that show either environmental persistence or a high potential to bioaccumulate.

Industrial chemicals are generally not deliberately released into the environment as broadly as pesticides. The presence of some industrial contaminants stems largely from their relatively high persistence or their potential for bioaccumulation. It may not be practical or necessary, therefore, to test all industrial chemicals for avian reproduction effects, although it was the opinion of some group members that all major use chemicals should be tested. The setting of exact triggers for non-pesticide compounds should be co-ordinated with current OECD efforts to define persistence and bioaccumulation.

For practical purposes, it is intended that this test should be performed on technical active ingredients, but the test may be used equally well on formulated products or mixtures should this be required.

Proposal for the Reproduction Test

Principles of the Test

Selection of test species

There was consensus that the Japanese quail is currently the most attractive species on which to conduct a reproductive study as described in this report. There are several advantages in working with this species, including a rapidly attained peak egg production level, allowing for greater test efficiency, and a short maturity time, allowing for the investigation of F1 effects should this be indicated. Should it be desirable to conduct a second test of reproduction, the mallard would currently be the logical choice. In addition, it was agreed that a test of reproduction that includes many of the behavioural aspects not currently covered in avian reproduction testing, probably with a passerine species, should be developed to allow the possibility of more realistic testing.

There was also consensus that more work is needed on comparing the output of reproductive studies performed to date. The comparison between bobwhite quail and mallard is currently the easiest to perform, in view of the very large database that already exists. However, given that the Japanese quail has been recognised to hold the highest potential as a test species, it is even more critical that the comparison of study results between the Japanese quail and the other two test species be carried out. There are several possible sources for comparative data. These data should be collated and analysed as soon as possible.

There was extensive discussion on which species should be used in tests for reproductive effects. It was recognized that the current tests do not, and cannot, model a realistic breeding cycle in birds. The three current test species (bobwhite quail, Japanese quail and mallard) were therefore as useful as any other birds in a tier 1 test of reproduction, as described here. The relative merits of the three species were considered in relation to the science of the tests and the practicality and cost of running them.

Current knowledge

There is an extensive database on bobwhite quail and mallard, since these birds have been used more than Japanese quail in testing, particularly for pesticide registration in North America. Because of their usefulness in comparisons between species and

among chemicals, these data should not be lightly discarded. Classes of compounds can also be compared using such large data sets. The Japanese quail is increasingly being used in tests in Europe and elsewhere in the world. The database on this species is therefore increasing, but has still not reached anything approaching the size of the database on the other species, especially for pesticides.

Advantages and disadvantages of each species

Mallard and bobwhite quail take months rather than weeks to reach sexual maturity, which limits their use in looking at effects on the F1 generation. Mallard and bobwhite quail require larger pens than the Japanese quail, the former because of their size, the latter because they are more 'flighty' and susceptible to self-inflicted injuries and cannibalism. Both mallard and bobwhite quail, but especially the mallard, show a much narrower plateau of peak egg production than the Japanese quail, which very quickly achieves and then maintains peak egg production levels. For example, the bobwhite quail does not reach peak egg production until weeks 6-7 of laying, lays at peak levels for approximately 4 weeks, and then starts declining (Collins 1994). The mallard reaches peak production faster (after approximately 3 weeks), maintains it for approximately 4 weeks but then shows a very abrupt decline (Collins 1994). The reason for the precipitous decline in egg laying is that the mallard, like the majority of avian species, shows a photorefractory period. This feature could be seen as an advantage for the sake of realism, but it presents serious practical problems in a standard test protocol. The Japanese quail reaches peak production after only 2-3 weeks and maintains this production at least for the following 6-8 weeks (Solecki et al. 1992, 1994). Therefore, with the mallard and bobwhite quail there are greater limitations on the ability to use pre-dosing performance of individual birds to compare with chemical effects on egg laying, fertility, hatchability, etc.

The Japanese quail, on the other hand, exhibits photoperiodic drift, i.e. the ability to grow the gonad on what would normally be considered non-stimulatory daylengths. This would be a disadvantage if the onset of egg laying were used as a major endpoint in tests, since birds would come in to lay over a variable time course. If this aspect is not tested, the Japanese quail has advantages over the other species in size, ease of housing, relative lack of aggression and 'flightiness', and in reliable egg production over a long period. The fact that this species has been bred for a high and consistent egg production has been of concern to several people. At this point, however, there is no evidence, or reason to suspect, that such selection has had an impact on the toxicological sensitivity of the species.

Other species

The use of a species able to undergo a full breeding cycle more closely resembling natural breeding under laboratory conditions also presents considerable attraction and was discussed. The current design of avian reproduction tests, as well as the design suggested in this report, preclude the testing of a variety of ecologically relevant endpoints. These endpoints would include:

- onset of egg laying under photoperiodic stimulation, which would be encountered at breeding in the wild;
- effects interfering with the bird's perception of environmental variables, such as food availability, nest site availability, weather, etc., all of which affect timing of onset of breeding in the wild;
- incubation effects which reduce egg hatching;
- parental behavioural effects which reduce effective rearing of chicks;

- exposure and effects on altricial young being fed by parent birds; and
- social interactions between individual birds and their position in social hierarchy.

Steps should be taken to design test protocols covering these aspects of reproductive testing in birds. The use of a passerine species such as the zebra finch (*Poephila guttata*) has been proposed, but studies have also been carried out in which mallards were allowed to incubate their own eggs. The use of altricial species does have the advantage that it allows observations to be made on parental care. There are problems to be worked out, however, such as the seed shelling habits of many birds, which makes the determination of ingested dose difficult to determine. The use of these species, however, should not be seen as an alternative to the present tests, but an adjunct which would provide extra information to risk assessors. The proposed test with the Japanese quail offers opportunities to measure, in detail, a variety of biochemical and physiological parameters which a naturally breeding colony of birds could not provide because of the risk of disturbing the breeding process.

Tier placement

There was consensus that a test of reproduction must be at the first tier of product testing and form part of the standard information package available for all pesticides proposed for registration.

A test of reproductive toxicity has historically been triggered by evidence of repeated exposure, undue persistence, or evidence of reproductive effects in mammalian systems. Products need not be persistent for birds to be exposed repeatedly because of multiple field applications of chemicals. Furthermore, reproductive impairments have been documented following short-term exposures to chemicals. In practice, it is difficult to predict exposure from chemical use patterns. Therefore, the precautionary principle requires that reproduction should be tested at the earliest tier.

Interspecies variation and the use of safety factors

There was consensus that the use of safety factors is appropriate in the context of reproductive endpoints, and that their use may eliminate the need to test several species. This was considered to be desirable from an animal welfare point of view.

There is no *a priori* knowledge that any of the currently tested species is more susceptible than another to reproductive effects caused by pesticides. Analyses of tests performed to date have begun, but do not yet provide sufficient information on relative species sensitivity. It has been suggested in the literature that testing a single species is insufficient (Mineau et al. 1994) because the two species currently tested, the bobwhite quail and mallard, showed that effects on hatchability and chick survival differed among pesticides. However, a different analysis (Schmuck et al. unpublished) indicates that there may be more similarity between the two species when overall chick production is used as the endpoint. These last results suggest that a safety approach factor could be used to account for species differences. There is no scientific reason to believe that the variability in reproductive response following chemical challenge is any less than the variability currently seen in lethality tests. On the contrary, the multiplicity of biochemical and physiological factors involved in the reproductive process suggests that the variability among species is in fact large.

To the Working Group's knowledge, a safety factor to account for interspecies variability is not currently applied to reproductive endpoints for the purpose of risk assessment in birds, although this approach is the norm in human health assessment. However, the avian reproduction study (whether current designs or the design proposed here) provides for an implicit safety margin by giving the birds a constant dose level which does not take into account field degradation of the pesticide.

Dose setting

The Working Group recommended that dose levels should be set so as to observe significant effects on reproductive output (e.g. egg or chick production), at least at the highest dose. An upper limit should be set at 1000 ppm or 2 X PEC, whichever is the greater.

Two different approaches to setting dose levels were considered. The first entails starting from an expected (predicted) environmental concentration (EEC or PEC), augmented by a safety factor. There are several disadvantages to relying on PECs to set dose levels. The first is that projected uses of chemicals may change over time, or information may become available which would dramatically change PECs. This has led to situations where dosing levels chosen for the test become inadequate, so that the test has to be repeated. A second problem is that different jurisdictions set PECs very differently, making harmonization and mutual acceptability of test results difficult. When no effects have been observed, there still is the need for an adequate margin between the PEC and the upper dose so as to account for interspecies variation. This margin is unlikely to be sufficient if dose levels are set on the basis of a PEC.

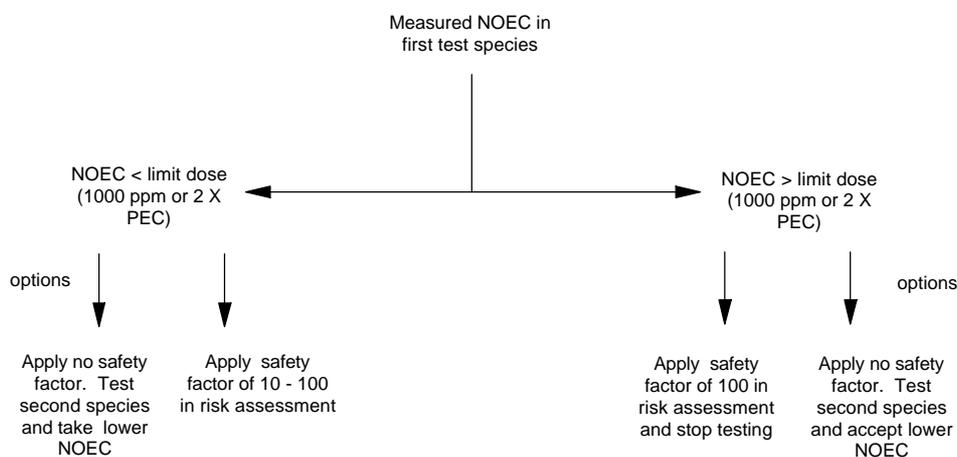
The alternative approach is more akin to the mammalian approach, which is to select a maximum dose to obtain an effect and use doses below this effect level to define NOECs. Such an approach would greatly enhance the mutual acceptability of data and increase the likelihood that dose levels would not be inferior to PECs, however calculated. It is appropriate in this approach to set upper dose limits beyond which testing is not appropriate for scientific as well as animal welfare reasons. The Working Group recommended an upper limit of 1000 ppm or 2 X PEC, whichever is the greater. The 2 X PEC rule is designed specifically to accommodate seed dressings or other situations where worst case estimates of PEC are extremely high. For animal welfare and scientific reasons, the dose levels chosen should be below those that cause severe parental toxicity.

Tier progression

The Working Group recommended progression of testing, starting with a first tier test in the Japanese quail with a choice of a second species or the application of safety factors.

Tier progression is outlined in **Figure 4.1**. At least one species, preferably the Japanese quail, should be tested at the first tier. If an effect on reproductive output is seen at a level below a limit level of 1000 ppm or 2 X PEC (predicted environmental concentration), whichever is higher, no other species need be tested. But the NOEC should be divided by a safety factor in order to account for interspecies differences in susceptibility. A factor of 10 would be appropriate based on the preliminary results of Grau et al. (unpublished), but this may be modified following finalisation of that work and/or receipt of new information. Alternatively, a second species should be tested and the one with the highest sensitivity used in the risk assessment.

Figure 4.1 Tier Progression in Avian Reproduction Test



If no effects on reproductive output are seen at or below a limit level of 1000 ppm or 2 X PEC, whichever is higher, two choices are open:

1. No other species is tested but a larger safety factor of 100 is applied. This is the precautionary approach, following the analysis by Mineau et al. (1994), which showed a discontinuous distribution of sensitivity between the mallard and bobwhite quail for some of the critical reproductive endpoints.
2. A second species is tested, and no safety factor need be applied to the results. Risk assessment proceeds using the more sensitive of the two species.

The option to test more species would always be open, in order to use weight of evidence information in coming to a credible risk assessment. However, it was recognized by most members of the Working Group that the avian reproduction test is only a rough screening tool, and that confirmation or negation of predicted hazard may require higher tier testing either in the laboratory or the field.

There was also general agreement that some of the endpoints measured in the proposed (or currently designed) avian reproduction study are not as meaningful as others. Earlier, it was advocated that most of the emphasis be placed on ecologically relevant endpoints such as the production of sexually viable chicks. Mineau et al. (1994) advocated separation of endpoints between parental, eggshell and developmental effects, arguing that the latter, in the absence of any visible parental toxicity, were of greater regulatory interest. Although the final decision probably needs to be made on a case-by-case basis, it has been suggested that endpoints such as food consumption, and possibly adult body weight as well, should not lead to the same tier progression as other endpoints.

There is currently a serious lack of data pertaining to the field validation of reproductive effects seen in the laboratory. This type of information exists for organochlorine compounds, but not for pesticides with a shorter environmental half-life. This should be considered a priority research topic.

Statistical power considerations

Eliminating a pre-laying dose period, utilizing proven breeders only, and using pre-dosing data as a covariate would result in greatly enhanced statistical power of the proposed test. However, a period of exposure pre-laying should be retained for substances that show the potential to bioaccumulate.

A rigorous power analysis of the current test design (Collins 1994) indicates that as many as 74 pairs per dose group are needed for bobwhite quail in an 8-week study to achieve 80% power of detecting a 20% reduction in the number of eggs laid. Current protocols require between 12 and 16 pairs per dose group. Practicality, expense, and animal welfare considerations preclude the use of sufficient numbers of birds to achieve adequate power.

In designing a new test of avian reproduction, the Working Group therefore wanted to improve the sensitivity and statistical power of the test to detect the important endpoints. The goal of increased power was to be met without significantly expanding the test. Test duration was also reconsidered in light of the current emphasis on the development of less persistent pesticides. In addition, it was felt important to include consideration of recently reported effects of chemicals with the capacity to disrupt endocrine systems, leading to loss of reproductive viability of the offspring.

The Working Group opted for a study design which allowed for the use of pre-dosing reproductive performance following stabilization of egg laying performance, to help ascertain chemical effects using each pair as its own control. The usual untreated control group was considered to still be required. A study design with analysis of covariance has increased power when compared to other designs. The Working Group believed that a study with two weeks of pre-dose egg production and at least six weeks of dosing and 20 pairs/dose may be able to attain 80% power to detect 20% reductions in performance. Further analysis is necessary in order to determine the exact number of adult pairs to be tested in each dose group, as well as the appropriate study duration.

The previous test protocols included a dosing period on short days prior to photostimulation. The original purpose of this pre-breeding period was to allow the build-up of residues of pesticides which bioaccumulated. With the onset of breeding induced by the lengthened day, these residues were likely to be released from fat and affect reproductive parameters. With the increasing development of pesticides which do not bioaccumulate and the phasing out of older compounds which still show this characteristic, the relevance of the short day exposure was questioned.

Endpoints potentially affected by the removal of a dosing period prior to photostimulation were discussed:

- the onset of egg laying (which could have been measured in previous protocols but was not often considered);
- effects on male fertility which might develop during this period.

Onset of egg laying was seen as a major endpoint of ecological relevance. Bird populations in the wild adjust the onset of lay to avoid locally adverse conditions when they reach physiological breeding condition. However, it was not thought possible to test these parameters using a basic test. The stimulatory photoperiod was inappropriate in the current protocols since excessive photostimulation effectively outweighs all other physiological responses to environmental variables. The test species commonly used in basic tests do not show the same physiological responses as wild birds. Caging conditions preclude the observation of most environmental variables which would need to be tested. It was therefore concluded that onset of egg laying, along with other such factors, could only be monitored in a more natural protocol with different test species.

Male fertility effects are currently a major concern. However, it was felt that any effects on male fertility would be seen in a 6-week period of dosing of breeding birds given the gametic cycle in the male testis, which has been estimated (in Japanese quail) to be approximately 25 days (Johnes and Jackson 1972) and that 2-weeks is the maximum period of viability for sperm stored in the oviduct, with fertility dropping quickly after 5-7 days.

In consideration of the above, the Working Group believed that the ability to perform covariance analysis outweighed any advantages to be gained from dosing prior to initiation of egg laying. Allowing birds to begin laying before dosing permits the identification and elimination from the test of any non-laying or infertile pairs, thus further increasing the statistical power of the test. Birds can also be stratified randomly by weight prior to treatment to equalize inter-group variance. An exception in this regard may be for substances that show the potential to bioaccumulate. For those substances, a period of exposure pre-laying should be retained.

Determination of effective dose levels

It was the consensus of the Working Group that, at present, NOECs should continue to be reported along with the statistical power of the test from which they were derived. In the longer term, the use of low-effect levels (e.g. EC_x values) to replace NOECs in risk assessment should be investigated.

The overall output of the test should be a no-observed-effect concentration (expressed in dietary terms since this is the route of exposure). This could be expressed for the overall endpoint, but also for the intermediate endpoints where these were affected by treatment. The weight given to intermediate endpoints in the absence of a problem in overall chick production is a case-by-case decision which must be made after consideration of the possible or likely consequences in the wild. It was acknowledged that a NOEC may be a poor basis for comparing different endpoints, or even different studies, when the statistical power of the test has not been explicitly stated. A NOEC may simply be a function of high intra-test variability and therefore not be toxicologically meaningful. It has been proposed that EC_x levels be computed instead where x refers to a chosen effect level relevant to the endpoint being measured. Notwithstanding the statistical strength of this argument, it was the consensus of the Working Group that NOECs should currently continue to be reported because the curve fitting necessary for the determination of an EC_x has not been attempted. One of the reasons is that there are few, if any, empirical data sets available to test this way of analysing test results. Furthermore, test designs currently used (e.g. with 3 dose levels) may not allow for adequate fitting. The power of the reproduction test should be reported, allowing an assessment of the validity of the NOEC.

In many cases, given the limited range of dose levels in the test, a no-effect concentration may not be seen. In these cases, extrapolation from a lowest-observed-effect level would be desirable both on scientific grounds and so as to reduce the need for another test, which is both a cost and animal welfare issue. A better knowledge of appropriate curve fitting techniques would therefore be desirable whether NOECs or ECx levels constitute the endpoints. There was consensus that this should be an immediate priority for research.

In determining an effect level for use in risk assessment, it was considered that the production of sexually viable 14-day chicks was the most important, for reasons outlined above, and that this endpoint should not be compromised. However, it was recognized that more information of potentially great value to understanding chemical effects in wild birds may be derived from intermediate endpoints. Moreover, from an ethical point of view, we should be striving to extract as much information as possible from the study, keeping in mind concerns over costs and unnecessary testing, especially if this information will alleviate the need for repeat or complementary studies utilising more animals.

Procedure

General aspects

Tests should allow for the collection of pre-dose data for a minimum of 2 weeks following peak egg production (expected to take about 3 weeks in the Japanese quail), followed by at least 6 weeks of dosing during which all eggs are collected and set. Analysis and reporting of study results should be standardised in order to facilitate comparison of studies.

Japanese quail reliably reach a plateau in egg production three weeks after initiation of laying, which makes this species ideal for the covariance analysis recommended in this document. For a 2-week period after egg production has stabilized, eggs should be collected and set in order to obtain data on all normal reproductive endpoints. Birds should then be assigned to treatment, and dosing should begin. Dosing should continue for at least 6 weeks and *all* eggs from the *entire* dosing period should be set.

A minimum of three dose groups should be tested, in addition to a control group. The setting of dose levels has been described earlier.

Analysis and reporting of study results should be standardised, in order to facilitate comparison of studies.

Measurements of main endpoints

The current suite of functional endpoints was considered to be acceptable, with minor modifications described below:

Feed and feed consumption:

The caloric value of the feed and its composition should be explicitly reported.

Although food consumption is currently measured, it is not possible to relate food (and therefore chemical) intake in this study to food intake in other dietary studies without accounting for the caloric and nutritional character of the feed. This measurement should also take into account the carrier (usually a vegetable oil) added to the diet in order to mix in the chemical. The amount of the carrier added to each dose group should therefore be constant.

Eggshells:

Measurement of eggshell breaking strength would be required, as well as the standard thickness measurement.

Eggs should be taken for eggshell measurements on a single day every two weeks as under the present guidelines. Measurement of the breaking strength of eggs using a recognised bench tester, in addition to eggshell thickness measured along the equatorial region, is more reliable than use of thickness alone. In some cases, even when thickness is not reduced, eggshell breaking strength may decrease. Treatment-related changes in eggshell quality may also increase the rate of eggshell breakage. On the other hand, egg breakage may be related to handling, inadequate animal management and caging, and/or behavioural anomalies in adults related to treatment or husbandry. Breakage (cracking) should continue to be recorded, but with the inclusion of caveats about interpretation. The eggshell strength and thickness parameters are more significant and more reliable. When sufficient experience with breaking strength has been gained, the measurement of eggshell thickness should be dropped to reduce unnecessary work.

Fertility and early embryo death:

Eggs not alive at first candling should be opened, to distinguish between infertility and early embryo death.

The most efficient way to handle the eggs is to perform one early candling and eliminate the second candling performed in previous protocols. Non-viable eggs should be opened at the first candling, to distinguish between infertility and early embryo death. The Working Group recognized that some mistakes may be made, with some live eggs withdrawn from incubation, but important information would nonetheless be gained by breaking the eggs out. Analysis of the results would have to take into account the erroneous removal of live eggs if this occurs.

Examination of F1 chicks:

Gross examination of the gonads, and sex determination of all chicks, should be performed at the high dose, with examination of other dose groups and histopathological assessments when effects are found. As an option, the reproductive viability of the F1 could be assessed directly by taking the birds to breeding age.

In addition to counting the number of healthy 14-day-old F1 chicks, the Working Group recommended that steps be taken to assess their reproductive viability. Testing the functional reproductive capacity of the F1 generation is too labour intensive to be recommended as part of a standard protocol. However, the option of allowing the F1 to reach breeding age is always there and the use of the Japanese quail, with its rapid sexual maturation, makes this feasible. The Working Group's opinion was that it is probably not

meaningful to measure hormone levels in young chicks. Use of this potential biomarker therefore does not hold much promise.

Microscopic examination of the reproductive tract of chicks should be performed if gross abnormalities, such as disparities of gonad size, are found. However, endocrine disruptions may not always result in gross abnormalities of the gonads. Therefore, evidence from other systems, such as endocrine abnormalities in mammals or *in vitro* systems, should also lead to microscopic examination of the reproductive tract.

Chicks from other dose groups should be examined if too few chicks remain in the high dose group for a reliable analysis.

Examination of adults:

Body weight should be determined just before initiation of dosing and at the end of the study. Clinical signs of toxicosis such as lethargy, depression, wing droop, ruffled feathers, etc. should be recorded at least once a day.

At the termination of the study, necropsy and assessment of gross pathology should be performed for all animals as recommended in the German proposed protocol submitted to OECD. Wet weight of liver, spleen and male gonad should be determined. Histopathology should be undertaken on organs showing gross pathological changes.

Animal Welfare

At this time, the Working Group considered that it is not feasible to assess *in vitro* the potential of chemicals to affect avian reproduction. Reproductive effects are considered important enough that testing should be carried out for all pesticides giving rise to avian exposure, as well as major use industrial chemicals which are either persistent or bioaccumulatory. This may result in an increase in testing over the present situation. However, the proposed scheme would make the use of a second test species unnecessary provided a safety factor is applied to the NOEC. The proposal to make the Japanese quail the main test species was also considered a positive step because this species is less susceptible to self-inflicted injury in captivity. The test design suggested here ensures that the power of the test can be maximized and the number of animals kept to a minimum. Finally, the Working Group proposed that the test be significantly shortened. Animals exhibiting severe distress will be euthanized in an approved way.

Need for Alternative Tests of Avian Reproduction

Surface Exposure of Eggs to Chemicals

It is impossible using the current test protocols to examine exposure of eggs and chicks from direct overspray, or the transfer from parental plumage, of chemicals. This route of exposure may be especially relevant in situations where light hydrocarbons or other chemicals capable of penetrating eggshells are present singly or in formulation. Egg spraying or dipping has been carried out by numerous investigators, and guidance is available (e.g. Hoffman and Albers 1984). The choice of species may be critical since

eggshell porosity and therefore interspecies susceptibility may vary. Mallard eggshells are more porous than quail eggshells, and mallard eggs are therefore considered to be more suitable than those of quail for testing. Passerine eggshells are thinner and may be more susceptible yet. Relative porosity could be assessed using the rate of moisture loss in eggs placed in dessicators to guide the choice of test species.

Short Exposure Test

To refine risk assessment, an additional test using shortened exposure periods which are more realistic for some pesticides available at high levels but rapidly degraded in the environment should be considered.

There was also some discussion of a test with a much shortened exposure period. This is because many pesticides are currently available at high doses for much shorter periods than the exposure periods used in the proposed test. Use of a shorter period may also be desirable in order to test the effect of higher concentrations of chemicals which cannot be given to birds over a prolonged period without incurring serious problems of parental toxicity. Interpretation of such a test is likely to be difficult. Much more discussion of this concept is needed. For example, a short chemical exposure in captivity may lead to a suppression of laying followed by a resumption of full egg production a few days later (Bennett, unpublished observations). This may seem a minor effect in the laboratory, but the resulting de-phasing of the breeding effort may have much more serious consequences in the wild. There was consensus, however, that a short exposure test should not replace the standard test described earlier but rather should be at a higher tier. Given that impacts of pesticides dosed over a short period at a higher dose may be a result of parental toxicity, a short exposure test might profitably be combined with a 'full breeding cycle' test in which parental behaviour is assessed.

Conclusions

The majority opinion of the Avian Reproduction Working Group was that a new avian reproduction test with the Japanese quail should be drafted following the various design elements listed above and submitted to OECD for consideration as soon as possible. *(Note from the Working Group Chair: The Working Group recognises that there is some opposition to moving away from the bobwhite quail as principal test species, but believes that, through analysis of existing data and/or further research, the many advantages of the Japanese quail and of new test design may be demonstrated).* The mallard reproduction protocol should be amended to reflect as many as possible of those same design elements.

The Working Group recommended that a test procedure be written for the Japanese quail for the reasons outlined above. A logical starting point would be the 1993 proposal by Germany, but this proposal would have to be radically changed to conform to the recommendations of this report. As a first step in this direction, several members of the Working Group met again in June of 1995 in Copenhagen to begin drafting a new protocol. Also, many of the approaches, as well as modifications to study endpoints, proposed by the Working Group for the Japanese quail may apply equally to the other species currently used in reproductive tests. Because a second species will often be required, the Working Group recommended that the current mallard study be rewritten to reflect as many of the design elements outlined above as possible. Because of the difficulty in preventing

Japanese quail from laying, the mallard might be the logical choice when testing bioaccumulatory substances requiring a lengthy pre-lay exposure. Some design elements proposed in this report may be less applicable to the mallard than to the Japanese quail (e.g. the use of the covariate approach may be limited by the short duration of peak egg production), and this will have to be considered further during drafting of the methods.

Recommendations for Further Work

It was also the consensus of the Avian Reproduction Working Group that the following research is needed:

1. An analysis of all available data on the relative sensitivity of mallard, bobwhite and Japanese quail to reproductive toxicants should be performed. If available data are insufficient to assess the relative sensitivity of these species, compounds identified as having caused reproductive effects in the mallard and bobwhite quail should be tested in the Japanese quail, possibly through incorporation in a ring-testing exercise. ***[Note from the Working Group Chair in light of comments received during the review of the draft Workshop report: This is considered to be a key requirement by those who oppose the move to the Japanese quail, and who propose that the bobwhite quail be retained as one of the principle test species. In order to make a convincing case for the Japanese quail as the preferred test species, those persons/organisations who have existing data on this species (primarily European industry) are encouraged to make this data available. It is also important that, if needed, proponents of the Japanese quail demonstrate a willingness to support the new protocol by generating the comparative data needed.]***
2. Ring-testing of the proposed basic Test Guideline for avian reproduction in the Japanese quail should be performed in order to evaluate its performance, sensitivity and reproducibility.
3. More work is needed on biomarkers for endocrine disruption, both in adults and in the F1. Measurements of mixed function oxidase (MFO) enzymes may also be useful, given recently documented correlations between elevated levels of some enzyme families and endocrine effects.
4. Statistical analysis is needed to refine the design of the avian reproduction test proposed here, so as to ensure maximum power while minimizing the number of animals tested. In addition, the Working Group recommends that appropriate statistical methods be established and made standard for analysis of all test results.
5. An additional test with a short-term exposure to doses higher than currently tested is needed for chemicals which are less persistent, and where parental toxicity may be expected to be high or dominant. This test would not replace the basic test recommended in this document.
6. Development of a full breeding cycle test in a passerine or other suitable species is required. This test is unlikely to replace the standard test, but might be used to augment information available to the risk assessor on effects of chemicals on natural incubation and hatching behaviour.

7. There should be field validation of reproductive effects seen in the laboratory.
8. Determination of predicted environmental concentrations (PECs) is also needed. Although a discussion of how PECs should be determined falls outside the mandate given to the group, determination of a PEC remains central to both the tier progression the Working Group is proposing and the interpretation of the results of the avian reproduction study.

Reading List and Literature Cited

Included in this list are the key documents considered by the Avian Reproduction Working Group in the course of their deliberations.

American Society for Testing and Materials (ASTM), 1990. "Standard practice for conducting reproductive studies with avian species." Vol. 11.04,6. American Society for Testing and Materials, Philadelphia, PA. pp. E1062–108.

Bennett, R.S., and Ganio, L.S., 1991. Overview of Methods for Evaluating Effects of Pesticides on Reproduction in Birds. U.S. Environmental Protection Agency. Corvallis, OR. **[Note:** Detailed critique of current US guidelines and suggestions for improvement.]

Burgat-Sacaze, V., Rico, A.G., and Petit, C., 1990. "Effets des pesticides sur la reproduction des oiseaux". Relation entre les traitements phytosanitaires et la reproduction des animaux, Ministère de la Recherche et de la Technologie, Paris. pp. 187-204. **[Note:** Very short review of avian reproductive problems seen with organochlorines and organomercurial compounds.]

Coenen, T.M.M., Brouwer, A., Enninga, I.C., and Koeman, J.H., 1992. "Sub-chronic toxicity and reproduction effects of Tri-*n*-butyltin oxide in Japanese quail." Archives of Environmental Contamination and Toxicology, Vol. 23, pp. 457-463. **[Note:** Companion study to the ring-test reported in Schlatterer et al. 1993. Important study - shows what was achieved by adding biochemical endpoints to the proposed German protocol.]

Collins, B.T., 1994. The avian reproduction study: Distribution of the control data and statistical power analyses. Canadian Wildlife Service Technical Report , Headquarters No. 214. **[Note:** Looks at power of current protocols by means of simulations performed on control data.]

Dobson, S., 1992. Assessment of the Relevance of the OECD Avian Reproduction Test. Project T07061a1, DOE Contract PECD 7/8/100, Institute of Terrestrial Ecology (Natural Environment Research Council), Monks Wood, Abbots Ripton, Huntingdon, Cambs PE17 2LS, pp. i&ii and 1-38. **[Note:** Places the current protocol in the context of avian reproduction in the wild.]

Jones, P., and Jackson H., 1972. "Estimation of the duration of spermatogenesis in Japanese quail, *Coturnix coturnix japonica*, using antispermatogonial chemicals." Journal of Reproduction and Fertility, Vol. 31 pp. 319-322.

- MacLeod, D.A., 1994. Statistical analysis methods for avian reproduction experiments. Canadian Wildlife Service Technical Report , Headquarters No. 211. **[Note:** Discusses how endpoints from current protocols are best analysed.]
- McLane, D.J., 1986. Hazard Evaluation Division Standard Evaluation Procedure. Avian Reproduction Test. Report EPA 540/9-86-139. U.S. Environmental Protection. **[Note:** Regulatory considerations associated with current EPA test guidelines.]
- Mineau, P., Boersma, D.C., and Collins, B., 1994. "An analysis of avian reproduction studies submitted for pesticide registration." *Ecotoxicology and Environmental Safety*, Vol. 29, pp. 304-329. **[Note:** A non-traditional analysis of studies performed according to current protocols. Includes consideration of interspecies comparison and bird-mammal comparison.]
- OECD. 1984. "206 - Avian Reproduction Test." OECD Guidelines for Testing of Chemicals. Organisation for Economic Co-operation and Development, Paris.
- OECD. 1994a. Draft OECD guideline for testing of chemicals. Avian sub-chronic toxicity test - oral toxicity (including effects on reproduction) in the Japanese quail following a 6-week administration in the diet. **[Note:** Annotated version of German proposal with comments from member countries].
- OECD. 1994b. Avian Reproduction Tests. Unpublished. 7 pp. **[Note:** This is a tabular comparison of 3 existing guidelines and the German guideline proposal.]
- OECD. 1994c. Member country comments regarding the inter-laboratory comparison study (document 5). Unpublished, 2 pp. **[Note:** Comments on unpublished version of Schlatterer et al. 1993.]
- OECD. 1994d. General comments on the German proposals for two avian toxicity test guidelines. Unpublished, 2 pp.
- Schlatterer, B., Coenen, T.M.M., Ebert, E., Grau, R., Hilbig, V., and Munk, R., 1993. "Effects of Bis(tri-*n*-butyltin)oxide in Japanese quail exposed during egg laying period: An inter-laboratory comparison study." *Archives of Environmental Contamination and Toxicology*, Vol.24, pp. 440-448. **[Note:** Important paper. Ring-test of the German proposed protocol.]
- Schmuck, R., Grau, R., Fischer, D., Ebert, E., Romijn, K., Brugger, K., and Munk, R., unpublished. Comparative analyses of adverse effect levels of pesticides on reproductive performance of Bobwhite quail and Mallard ducks. Preliminary draft. **[Note:** A summary of this study was distributed at the Workshop.]
- Solecki, R., Hilbig, V., Pfeil, R., Gericke, S., and Gottschalk, M., 1992. "Bis-(tri-*n*-butyl-zinn)-oxid: Vergleichende Prüfung des Einflusses von Einzel- und Paarhaltung auf die subchronische orale Toxizität unter Einbeziehung reproduktionstoxikologischer Parameter nach 13wöchiger Verabreichung über das Futter und nach 3wöchiger Nachbeobachtung an der Japanischen Wachtel (*Coturnix coturnix japonica*)". Auftrag des Umweltbundesamtes, Berlin. **[Note:** This and the next internal UBA report present raw pen by pen information on breeding studies using Japanese quail. The reports were not available to the Working Group but the information was relayed by Dr. Solecki and will form part of the database to be analysed as per recommendations of the Working Group.]

- Solecki, R., Hilbig, V., Pfeil, R., Gericke, S., and Schellschmidt, B., 1994. "Parathionmethyl: Prüfung der subchronischen oralen Toxizität unter Einbeziehung reproduktionstoxikologischer Parameter nach 6wöchiger Verabreichung über das Futter und einer 3wöchiger Nachbeobachtungsperiode an der Japanischen Wachtel (*Coturnix coturnix japonica*)". Auftrag des Umweltbundesamtes, Berlin.
- U.S. Environmental Protection Agency (U.S.E.P.A.) 1982. Pesticide Assessment Guidelines. Subdivision E, Hazard Evaluation: Wildlife and Aquatic Organisms. Report EPA-540/9-82-024. U.S. Environmental Protection Agency.
- U.S.E.P.A. 1995. Ecological effects test guidelines. OPPTS 850.2300. Avian reproduction test. EPA 712-C-94-141. [**Note:** Protocol for the Bobwhite quail harmonized between U.S. TSCA and FIFRA legislation. Distributed as pre-publication draft at the Workshop.]
- WHO. 1984. Principles for Evaluating Health Risks to Progeny Associated with Exposure to Chemicals During Pregnancy. Environmental Health Criteria 20. World Health Organization, Geneva. [**Note:** Slightly dated but useful compendium of the strategies employed in the assessment of mammalian reproduction.]

5. Testing for Avoidance

Introduction

This section presents the conclusions of the Working Group on Avoidance. The format is intended to be suitable for development into a guidance document on avoidance testing for birds. Each sub-section contains a brief account of the arguments considered by the Working Group and the conclusions reached. **This report represents the consensus view of the group, except in the few places where differences of opinion are noted. In the sections on Initial Considerations and Principles of the Tests, the key conclusions are printed in bold.**

Initial Considerations

Potential Role of Avoidance Testing in Avian Risk Assessment

In the wild, a range of different foods and habitats are available to birds. They may avoid those which are contaminated with toxicants. There are many examples of wild birds avoiding foods which contain natural chemicals whose repellency has been confirmed in tests with captive birds (e.g. Brower and Fink 1985, Buchsbaum et al. 1984, Crocker and Perry 1990, Jakubas and Gullion 1990, Kvittek 1991, Mason 1990, Mason et al. 1989b, Mason et al. 1991, Mason and Turpin 1990, Rowell-Rahier et al. 1995). Many pesticides are strongly avoided by captive birds in the 5-day dietary toxicity test (see consumption data in Hill and Camardese 1986) and in other types of test (e.g. Avery 1989, Avery and Decker 1991, Avery et al. 1993, Avery et al. 1994a, Avery et al. 1994b, Babu 1988, Bennett 1989a, b, Bennett and Prince 1981, Grau et al. 1992, Grue 1982, Hill 1972, Kononen et al. 1986, 1987, Mason and Reidinger 1982, Robel and Morrow 1987, Rogers 1974, Schafer et al. 1983, Schafer and Brunton 1971). Furthermore, adding repellents to pesticide formulations may cause them to be avoided by birds (Mastrota and Mench 1995). **It therefore seems reasonable to suppose that some pesticide products may be avoided to some extent by birds in the wild, reducing the risk of poisoning below what would otherwise be expected. Thus tests of avoidance may have a role in the later stages of risk assessment, as one of the factors to be considered in refining the preliminary assessment of risk.**

Terminology

Several terms have been used in relation to the phenomenon of avoidance, including *repellency*, *palatability*, *acceptance*, *anorexia* and *aversion*. Most of these terms imply particular mechanisms, or are not applicable to the avoidance of materials other than foods. **The group used 'avoidance' as a general term**, but did not debate terminology in detail.

Existing Methods for Testing Avoidance

Testing for avoidance has been requested in the past for some pesticides by regulatory authorities in France, Germany and the UK. Guidelines have been published in

France and Germany (INRA 1990, BBA 1993). In addition, a number of approaches have been proposed in the scientific literature for routine use (e.g. Schafer et al. 1983, Bennett and Schafer 1988, Grau et al. 1992, Kononen et al. 1986, Kononen 1988). Luttik (1993) suggested that the standard 5-day dietary toxicity test (LC50, e.g. OECD 1984) could be modified to provide a measure of avoidance as well as toxicity. However, it has also been argued that various shortcomings of this test make it a poor guide to exposure and toxicity in the wild (Mineau et al. 1994).

A variety of methods have been used for testing the efficacy of chemicals for use as repellents to protect crops and other areas from birds. These methods include molecular modelling, cage tests, large pen trials and field studies (e.g. Avery 1989, Avery and Decker 1991, Avery et al. 1993, Clark and Shah 1991, Cummings et al. 1991, Mason et al. 1989a, Schafer and Brunton 1971, Starr et al. 1964). Many of the same principles apply to testing the efficacy of repellents and the ecological safety of pesticides, although there are some significant differences. Perhaps most importantly, a repellent need only be effective against one or a few target species whereas, for a pesticide, the potential for avoidance may need to be considered for a wide range of non-target species. Generally it will not be practicable to test more than a few species.

Are Tests with Captive Birds Reliable Predictors of Avoidance in the Wild?

Whether avoidance is effective in protecting birds from pesticides in the wild, and whether it can be reliably predicted from any type of test with captive birds, are matters of controversy. These questions need to be considered before deciding what type of avoidance tests, if any, should be used for risk assessment.

In a critique of the 5-day dietary test, Mineau et al. (1994) argued that, for a few relatively well studied cholinesterase-inhibiting pesticides at least, avoidance in laboratory tests is not borne out in the wild. The Working Group discussed these examples and others, including methiocarb (Dolbeer et al. 1994), bensultap (Edwards et al. 1993), imidacloprid (Grau et al. 1992, Avery et al. 1994a) and fonofos (Hart and Clook 1994, Hart et al. unpublished data). The group concluded that only for methiocarb, used as a bird repellent on fruit, was there strong evidence of avoidance being effective in reducing risk to birds in the wild. A large number of field studies showed that this use of methiocarb caused very few bird mortalities, and that birds took significantly less of treated than of untreated crops. Even in this case the effect of avoidance in reducing risk might be relatively small, since the predicted risk would not be very high even if there were no avoidance (birds feeding exclusively on freshly-sprayed fruit would ingest approximately one lethal dose per day based on acute toxicity).

In at least one case, strong avoidance shown by captive birds failed to protect wild birds. Diazinon has a high acute toxicity to birds, but is strongly avoided in dietary toxicity tests. In small pen tests where Canada geese were held on treated turf there were no mortalities, suggesting that avoidance was effective in reducing risk. However, a number of poisoning incidents involving waterfowl have been reported following the use of diazinon on turf and in field studies. A possible explanation is that the birds involved in these incidents fed more rapidly than captive birds and consequently ingested lethal doses before developing an avoidance response (Mineau et al. 1994). It may be possible to design a test which takes such effects into account.

The group felt that none of the other case studies provided enough evidence to decide whether risk was substantially reduced by avoidance in the field. In several cases,

where mortalities had occurred in the field, a number of factors contributed to uncertainty. Often the unrealistic conditions of laboratory studies (and pen trials) may have made avoidance more likely than it would have been in the field (e.g. using well fed birds, or a highly nutritional food base). There was also doubt as to whether the numbers of poisoning incidents in the wild were as high as would be expected if avoidance were having no effect at all in reducing risk (e.g. dieldrin and heptachlor - see Murton and Visozo 1963). On the other hand, where mortalities had not been recorded, factors other than avoidance (e.g. pesticide residues lower than expected) might have been responsible for the reduced risk. Moreover, mortality may have been underestimated due to poor search efficiency in field studies or under-reporting of poisoning incidents. In very few cases had an attempt been made to measure avoidance behaviour directly in the wild.

A comparison was presented between results from avoidance tests using the BBA protocol and the occurrence of poisoning incidents in the wild for the same pesticide formulations (a range of anonymous granular pesticides, treated seeds and baits; see Appendix to this section). Poisoning incidents had been reported for some but not all the formulations which had caused mortality in BBA tests. No incidents had been reported for formulations classified as low risk based on the BBA test. These data suggest that the BBA test provides a conservative measure of risk, although it should be noted that some formulations have been little used (so there has been little opportunity for risk to be realised) and that only a small fraction of incidents are reported.

The group concluded that considerable uncertainty remains about the extent to which avoidance is effective in reducing risk in the wild. A more detailed review of existing data is required to further assess the relationship between avoidance in laboratory and field. This might best be carried out by an OECD/SETAC working group, as some of the data are commercially sensitive.

Factors Which Can Affect the Extent of Avoidance

A wide range of factors have been shown to influence the extent to which treated food is avoided by captive birds, including species (Espaillat and Mason 1990, Kononen et al. 1986, 1987, Mason and Bonwell 1993, Mason et al. 1993, Schafer and Brunton 1971, Schafer et al. 1983), sex (Espaillat and Mason 1990), age (Williams, Fairbrother and Sullivan, unpublished data), group size (Kononen et al. 1986), social interactions (Mason and Reidinger 1982), previous experience (Greig-Smith 1987, Starr et al. 1964), type of treated food and prior food deprivation (Thompson et al. 1981), colour of the treated food (Greig-Smith and Rowney 1987, Mason and Reidinger 1983), the type of untreated alternative food available (Avery et al. 1995, Rogers 1974), the number of choices available (Bennett 1989a, b), ambient temperature and, possibly, the perceived risk of predation (Avery et al. 1994a). Some species feeding on treated seeds may greatly reduce their intake of chemical by removing the husks (Avery et al. 1994a).

This strong context-dependency implies considerable uncertainty in extrapolating from the avoidance responses of captive birds to the behaviour of wild birds. It was concluded that the reliability of the extrapolation should be improved by using test conditions close to those in the wild, rather than using a standardised test design. One member of the group disagreed, feeling that existing standard test guidelines and surrogate species had proven sufficiently reliable for regulatory purposes in the past. **Where a range of conditions may apply in the wild, the group considered it preferable to select those which are likely to reduce the extent of the avoidance response, to ensure a margin of safety in the subsequent risk assessment. That is, the test**

conditions should be realistic but tending towards the worst case. For example, choices between treated and untreated food could be made difficult rather than easy, and birds could be placed under at least moderate hunger stress. Balancing the need for realistic test conditions against the need to minimise suffering by test animals was a factor in the group's discussion of possible test designs (see later).

Should Avoidance Be Considered in Avian Risk Assessment?

In the light of the above considerations, the group decided that despite the uncertainty which still remains concerning the extent to which avoidance is effective in the wild, and the difficulties of extrapolation from tests with captive birds, avoidance should be considered in avian risk assessments for the following reasons:

- There are many examples of birds avoiding foods which contain natural chemicals whose repellency has been confirmed in tests with captive birds;
- Many pesticides are strongly avoided by captive birds;
- If the effects of avoidance were disregarded, potentially valuable products might be unnecessarily refused registration due to over-estimating risks to birds;
- Tests of avoidance can be made realistic and conservative to reduce the chance of over-estimating its effect on risk;
- Both test design and interpretation of results can be improved by making better use of information on the ecology and behaviour of the species at risk;
- Although avoidance may be partial, variable and context-dependent, these factors may be taken into account in assessing its effect on risk.

Chemicals Other than Pesticides

The group felt that the principles of avoidance testing for pesticides could equally be applied to other chemicals, taking into account the manner in which birds encounter them in the wild (e.g. in contaminated plant material, in other food, or in non-food particles; see later).

Proposals for Avoidance Tests

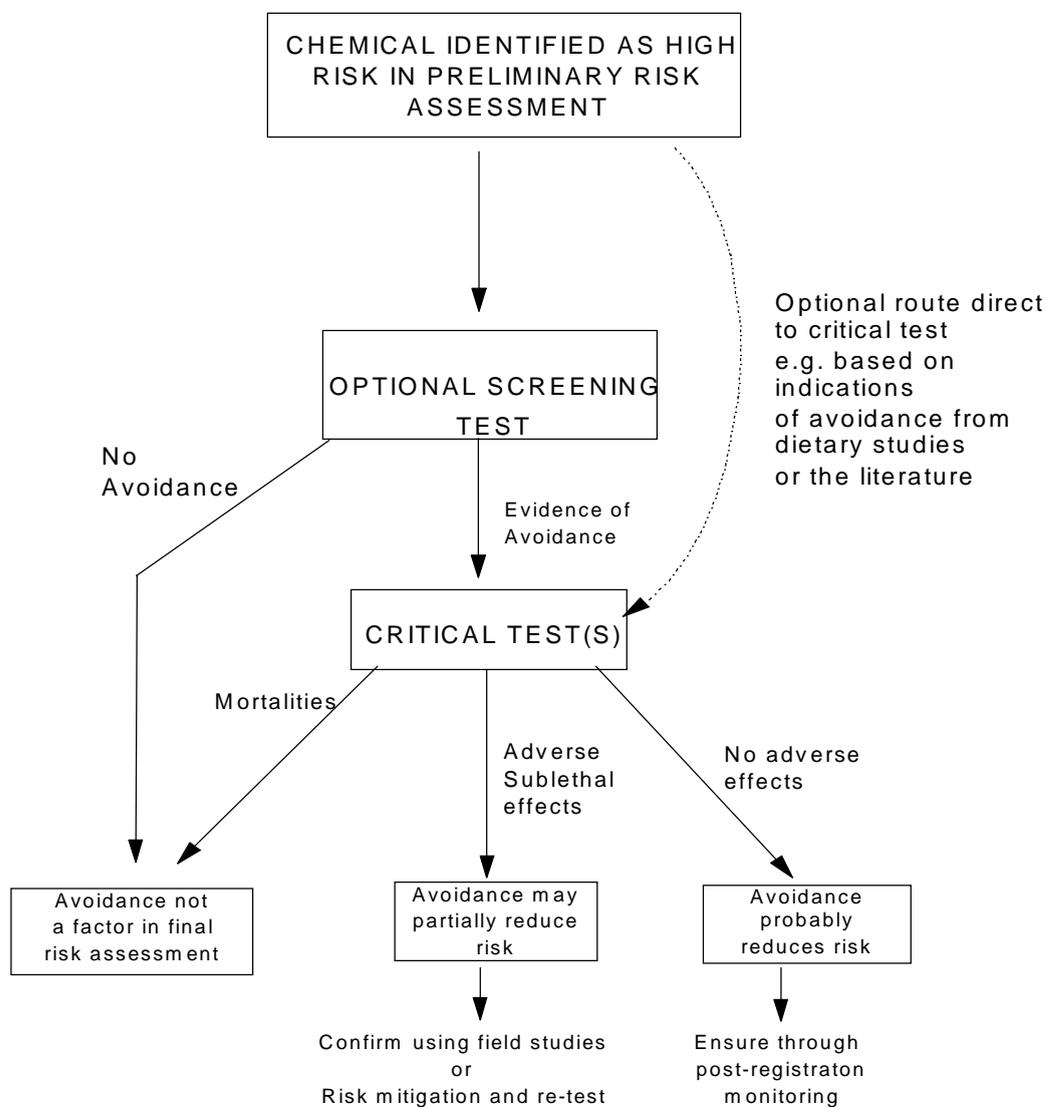
Principles of the Tests

Sequence of testing

Tests of avoidance would normally only be considered for pesticide uses for which a medium or high risk has been identified in a preliminary assessment. Usually, the concern would relate to one or more scenarios (crop, pest, manner and rate of

application, species at risk) which have been fairly closely defined. The sequence by which avoidance testing might proceed is illustrated in **Figure 5.1**.

Figure 5.1 Flow diagram illustrating proposed sequence for conducting and interpreting tests of avoidance



For reasons discussed above, **the final assessment of how avoidance may reduce risk should be based on a realistic and severe test.** The group referred to this type of test as a *critical* test. Because each test will be relevant to a relatively limited range of conditions, two or more critical tests may be required to cover the range of scenarios which have led to the preliminary assessment of high risk.

It may often be desirable to conduct a second type of test before the critical test. This was referred to as a *screening* test. A screening test would be simpler, less severe and less realistic than a critical test. **Its main purpose would be to provide a**

simple and sensitive test of avoidance in order to determine whether it is worth proceeding to the critical test. As such, the screening test was regarded by the group as an *optional* test, to be conducted at the discretion of the registrant. The registrant might prefer to proceed directly to the critical test if, for example, there is already evidence of avoidance in dietary tests, if the chemical is similar in structure to known repellents, or if the physical properties of the formulation are thought likely to cause avoidance. Equally, if a dietary toxicity study has already been conducted and shows no evidence of avoidance, it is unlikely to be worth proceeding to a critical test unless the properties of the formulation are expected to be different.

Screening tests are used in a similar way in the preliminary assessment of chemicals for use as bird repellents (e.g. Avery and Decker 1991, Mason et al. 1989). As realism is less important for the screening test, it is possible to adopt a more standardised method, which will aid comparison with other chemicals.

Even if some information on avoidance is already available from a standard dietary test, a screening test may still be useful for investigating the speed of onset of avoidance and whether avoidance is due to primary repellency or other mechanisms, such as toxicosis or conditioned aversion. This information can assist in refining the risk assessment (comparing time to avoidance with time to ingestion of lethal dose), interpreting the results of the standard dietary study, or interpreting results of the subsequent critical test (due to the lack of data on ingestion, or if the critical test has been performed with fewer treatment levels; see later).

Flexibility versus standardisation

The conclusion that the critical test should be realistic rather than standardised implies that if the species and situations differ between countries, tests conducted for one regulatory authority may not be acceptable to another. It would also be inappropriate to draft prescriptive guidelines for the conduct of critical tests, although this could be done for the screening test as it is more suitable for standardisation. The difficulty in developing standardised tests limits the progress which can be made towards OECD countries' objectives of harmonization and mutual acceptance of data, so far as avoidance testing is concerned. Further research is therefore urgently required to improve our understanding of the factors affecting avoidance, and thus our ability to extrapolate between different conditions. Improved understanding should make it possible to develop and validate more standardised methods, at least for particular types of formulation.

Nevertheless, greater harmonization of the general approach to avoidance testing should be possible. The group agreed that, for this purpose, it would be desirable to work towards the production of a Guidance Document identifying the options which exist for this type of study and discussing the rationale for preferring some options over others. It was hoped that, despite the need for further research on some issues, the contents of this report would be of immediate practical use both to regulators and registrants. In particular, it was hoped that those designing avoidance tests might find it useful to consider the proposals in each section below and incorporate relevant aspects into their own study protocols.

Need for prior information, expertise and consultation

The many factors which have been shown to influence avoidance by captive birds (see earlier) make the task of designing and interpreting appropriately realistic and severe tests a difficult one. Therefore the group recommended that, whenever such tests are contemplated, **advice should be sought from scientists with relevant specialist knowledge and experience**. It is recognised that such expertise will not always be available from the existing staff of either registrant or regulator.

For the same reasons, a **detailed dialogue between regulators and registrants is essential**, especially in the planning stage. Concern was expressed that such dialogue was often not practicable. Nevertheless, the majority view was that regulators and registrants should seek to agree, before the test is conducted, that the test design is appropriate and that, providing no unexpected complications arise, both will accept the results for use in risk assessment.

Also for the same reasons, the group agreed that a range of detailed information would be desirable to assist the design of the test (**Table 5.1**).

Table 5.1 Types of information which may assist the designing of appropriate tests of avoidance

Type of information	Possible sources
Acute avian toxicity of pesticide	Basic data set
Pesticide formulation	Basic data set
Crop, habitat, time of year, weather	Preliminary risk assessment
Species at risk	Preliminary risk assessment
Manner of exposure (food type/granules, etc.)	Preliminary risk assessment
Pesticide residues in food or other material	Preliminary assessment (e.g. 'Kenaga nomogram'), preferably augmented by data on actual residues
Pesticide half-life in appropriate media	Other parts of registration data package e.g. safety to consumers
Ecology and behaviour of species at risk, e.g. typical diet, foraging range, special factors (e.g. migrants)	Wildlife experts and scientific literature

Before conducting a critical test, an attempt should be made to estimate whether the birds at risk are likely to ingest a lethal dose of the pesticide before the onset of severe symptoms, such as would preclude further feeding. If this appears likely, then it may be best to conclude that avoidance will not be effective in protecting the birds and to forego the critical test, unless strong primary repellency (e.g. taste aversion) is demonstrated in the screening test. The calculation requires an appropriate LD50, information on the time it takes for severe symptoms to develop, an estimate of the maximum levels of residues to be expected, and estimates of the maximum rate of feeding. A calculation of this sort was successful in predicting the occurrence of poisoning incidents involving waterfowl feeding on diazinon-treated turf, whereas laboratory and semi-field experiments indicated avoidance (Mineau et al. 1994).

Description of the Screening Test Method

Objective

To provide a simple and sensitive measure of avoidance, with the primary purpose of determining whether the active ingredient is sufficiently repellent for proceeding to critical test to be worthwhile.

Test design

Priority is given to sensitivity, measurement of consumption, standardisation and economy, rather than to realism and severity (which are the priorities in the critical test, see later).

The group considered that the screening test should be conducted with a technical active ingredient, mixed into standard laboratory diet. Birds should be offered a choice between treated and untreated food, presented in cups or hoppers, with consumption of each being measured. A range of concentrations should be used to enable detection of the level below which the birds do not discriminate between treated and untreated food ('discrimination threshold'). If it is desired to use the screening test to investigate the speed of onset of avoidance, or the mechanism of avoidance, then more detailed measurements may be required. These could include more frequent measurement of consumption, continuous recording of behaviour, or physiological measures.

Test subjects

- To aid standardisation, the screening test might be conducted with surrogate species commonly used for LC50 and LD50 tests, such as the northern bobwhite (*Colinus virginianus*), Japanese quail (*Coturnix coturnix japonica*), or mallard (*Anas platyrhynchos*). However, consideration should be given to whether these are representative enough of the species at risk in the wild. Red-winged blackbird (*Agelaius phoeniceus*), European starling (*Sturnus vulgaris*), feral pigeon or rock dove (*Columba livia*), ring-necked pheasant (*Phasianus colchicus*) and brown-headed cowbird (*Molothrus ater*) have also frequently been used in pesticide studies, and may often be more representative.
- Age and sex of screening study subjects were not discussed in detail, although they may influence avoidance responses. It may be appropriate to use adults (as they are more likely to be exposed in the wild) of both sexes to provide the opportunity to detect a substantial sex difference if it exists.

Accommodation (housing)

- Birds should be housed singly to facilitate the measurement of individual food consumption.
- Birds in different cages should be visually isolated from each other to minimize social interactions that might violate assumptions of independence in the statistical analysis of the results.
- Isolation may not be acceptable in species in which this would cause undesirable additional stress (e.g. highly social species).

Preparation of animals

- A 5 to 7 day acclimation period to test conditions, or longer if required to stabilise daily food consumption, was recommended.
- After acclimation, a pre-treatment period equal to the test period, in which daily consumption of untreated diet should be measured, was recommended. This measure may be used to stratify the random allocation of birds to treatments, and/or as a covariate in analysing consumption data from the treatment period (see later).

Test material

- The test material in the screening test would usually be the technical active ingredient (ai), to provide the basis for extrapolating to different formulations. If it is considered likely that a particular formulation would have very different properties, then that formulation might be tested separately.
- Test material should be mixed into the standard diet, as used in the acclimation and pre-treatment periods.

Screening Test Procedure

Treatment levels

- The group considered that at least five concurrent treatment levels should be included in the test in order to obtain a dose-response curve. This curve could then be used to assess the degree of avoidance expected for a range of formulations or uses resulting in different environmental concentrations of the pesticide.
- The lowest treatment should be below the level expected to cause observable symptoms of intoxication. A negative control with two cups of untreated food would not be necessary.
- As the priority is to estimate the lowest level at which discrimination occurs, the highest treatment should be above this threshold unless it exceeds the maximum birds are likely ever to encounter in the wild. If it is desired to estimate the discrimination threshold with greater precision, it will be necessary to space the treatment concentrations closely around it, which will require a preliminary range-finding study.

Fasting

- Birds should be fasted for an appropriate period every night to simulate normal diurnal restrictions on feeding activity. This also increases motivation for feeding in the early part of the feeding period (Rogers 1974), ensuring measurable consumption and reducing inter-individual variation.

Duration of treatment and post-treatment periods

- A treatment period of 4 days should be adequate to allow detection of delayed learning or, in the case of an early response, habituation.
- Birds should be maintained on untreated food for a post-treatment period of 4 days to allow for observation of any delayed symptoms of intoxication, or longer if the test material is thought to be capable of causing more delayed effects.

Early termination of test on welfare grounds

- In the interests of animal welfare, the test should be terminated early if at any point sufficient mortality or other adverse effects have occurred to enable the regulatory decision to be made (i.e. to demonstrate that avoidance should not be a significant factor in risk assessment; see later).
- In addition, it would be desirable to remove individual subjects if they become moribund or suffer convulsions.

Presentation of test material

- Because of the priority given to accurate measurement of consumption in this test, feed should be presented in food cups or trays designed to minimise spillage and prevent exchange of treated and untreated diet.
- Each container should be filled daily with at least one day's feed requirement (e.g. approximately 25 g for adult bobwhite quail), so that birds could potentially obtain their full daily requirement from a single container.
- In many cases birds will exhibit preferences between containers, based on their position in the cage. It has been common practice to allow for this by switching the positions of treated and untreated containers each day (and making the treatment period an even number of days). It was reported to the group that, from a statistical point of view, it was preferable to allocate container positions at random. However, this may not be necessary in tests of up to 4 days, as the birds will not have time to learn the alternating sequence. The group concluded that until general guidance was available, those conducting tests should consult a statistician on this point.
- If it is desired to distinguish primary repellency and conditioned aversion, the feeding period may be divided into two parts. The choice of treated and untreated diet would be provided only in the first part, which should be long enough to ensure intake of an intoxicating dose at the highest treatment level if no avoidance occurs. Only untreated diet would be provided for the second part of the feeding period. Consumption and behaviour would be recorded separately for each part of the feeding period. Conditioned aversion might also be assessed by measuring intake of untreated diet in the post-treatment period.

Choices

- Because priority in this test is given to sensitivity rather than severity, birds should be provided with a free choice between treated and untreated food.
- No other cues should be provided as to the identity of the foods, i.e. there should be no difference in container or food other than presence of the test material. This implies that any carrier or diluent used with the test substance should also be applied to the untreated food.

Measurements

Measurements which should be made in the screening test include body weight, feed consumption, mortality, sub-lethal effects, environmental conditions, and residue in feed (**Table 5.2**)

Table 5.2 Measurements which should be made in the avoidance screening test

Measurement	When measured
Body weight	End of acclimation/beginning of pre-treatment; end of pre-treatment/beginning of treatment period; end of treatment period (all at the same time each day)
Consumption of untreated and treated food, corrected for spillage	Daily during acclimation period to assess stabilisation of intake; once or twice daily in treatment period; during post-treatment period if required
Mortality	As it occurs; especially note exact day and time and cause of death, as revealed by appropriate post-mortem investigations
Sub-lethal effects	Signs of overt toxicity and behavioral changes such as ataxia, wing droop or general debilitation as they occur, plus more detailed measurements if required to assess mechanism of avoidance
Residue in feed	At intervals sufficient to detect chemical deterioration
Environmental conditions (including temperature and daylength)	Daily (minimum and maximum temperatures)

Endpoints

- The primary endpoint is relative consumption of treated and untreated food. Appropriate statistical means should be used to represent the endpoint, but this topic was not discussed in detail by the Working Group. Kononen et al. (1986) proposed a log-probit analysis to estimate the food avoidance concentration (FAC50), defined as the highest dietary concentration that will result in equal consumption of treated and untreated food. Bennett and Schafer (1988) identified two shortcomings of this approach and proposed instead a two-phase regression analysis to calculate a discrimination threshold (DT), defined as the level above which test animals will decrease the proportion of treated food they consume if untreated alternatives are available. Luttik (1993) showed that these values were positively correlated with a no-repellency concentration (NoRC), which he derived from consumption in LC50 tests for a limited range of chemicals and species.
- The absolute amounts of feed consumed should be examined, as well as the ratio of treated to untreated, to check for generalised reductions in consumption (anorexia, Grue 1982).

- Mortality and sub-lethal effects are secondary endpoints which may help assess the mechanism of avoidance, provide information for comparing time to avoidance with time to ingestion of lethal dose, and reveal whether avoidance is insufficient to prevent mortality even in the favourable conditions of the screening test.

Statistical aspects

- Those conducting tests should obtain expert statistical advice on both the experimental design and data analysis, particularly regarding the numbers of animals required, the spacing of treatment levels, methods for estimating the endpoint (see above), and the analysis of changes in response over time.
- The number of birds to be used will depend on the degree of sensitivity required. The group was advised that if the test only involves one test concentration compared to a control, 5-8 replicates (individually caged birds) per treatment should be adequate. If, as will be more usual, there are 5 or more treatment levels, then it may be possible to reduce the number of birds per treatment.
- To improve precision and reduce the numbers of animals required, pre-treatment data on individual differences in normal daily consumption may be useful to stratify the allocation of birds to treatments, and as a covariate in data analysis. Allocation of birds would be more important for comparisons of absolute consumption than for the ratio of treated to untreated food consumed; a statistician should be consulted for guidance.

Description of the Critical Test Method

Objectives

To test whether mortality or adverse sub-lethal effects occur when birds are exposed to the pesticide in realistic conditions, with the purpose of deciding whether risk will be reduced to an acceptable level by avoidance in the wild.

Test design

For reasons discussed above, the group considered that the critical test should be realistic and severe. The requirement for realism implies that the test design must be very flexible, and that the results will be specific to a fairly narrow range of conditions. Expert knowledge and close dialogue between registrant and regulator are essential (see earlier).

In general, the study should be designed to offer birds the test material spread on the floor of a sufficiently large aviary, in a manner which reflects the exposure scenario of concern, for the duration of natural daylight over 4 days. Up to 4 treatment concentrations may be tested, plus a control treatment. Formulations should be used, not technical active ingredients. Positive and negative control treatments using other pesticides could be included to confirm that the test conditions are capable of distinguishing hazardous compounds which are avoided from those which are not. The primary endpoints should be mortality and sub-lethal effects. It would be desirable to measure consumption, but this may often be impracticable if test and alternative foods are presented in a mixture. A pre-treatment phase would be needed to identify the level of diet to be provided in the

treatment phase, to acclimate the birds to the diet and, if consumption is to be measured in the treatment phase, to provide a pre-treatment measure of consumption.

The group identified 3 broad types of exposure scenario for which radically different variations of this general design would be required:

- **non-food items** including most granular formulations: this may also include coated or pillorized seeds if these are reliably treated as non-food items by birds;
- food items that are difficult to handle in a laboratory diet, such as growing plants (**foliage**);
- **other food** including treated seeds and baits and natural foods other than growing plants.

Non-food items

These are materials with negligible nutritional value that are likely to be ingested incidentally or as grit. Note that granules which have some nutritional value (e.g. those with a corn cob base) should not be included in this category. During the treatment phase, birds should be presented with the test material spread on a soil surface. An adequate ration of suitable food would be spread on the same surface.

Foliage

Growing plants could become contaminated by spray applications, and may be ingested intentionally by birds. Pen trials are foreseen as the required method of testing, owing to the difficulty of bringing sufficient fresh leafy material into the laboratory. A pen would be brought to the treated field and placed over a test site, and birds would be introduced to the pen for feeding (e.g. Fink 1979, Robel and Morrow 1987). It must be noted that there are doubts about these tests' ability to represent birds' responses in the wild (e.g. for diazinon, Mineau et al. 1994). There is a need for research to refine and validate this method.

Other food

Other food types may be brought into permanent aviaries for testing. Treatment groups would receive a mixture consisting of several parts treated material (i.e. the food material that will be encountered in the field) and 1 part alternative food. The alternative food would either be the same food but untreated (e.g. seeds, insects, fruit), other foods, or a standard aviary diet (e.g. baits), to reflect the choices available in the wild. The untreated alternative would be offered at an amount below normal intake (about 75% of normal) to increase the severity of the test, to promote sampling by the birds, and thus to ensure that at least some treated food is taken.

For granules, treated seeds and pelleted baits the resulting design is similar to the BBA protocol 25-1, but with more treatment levels and other modifications that increase its realism. For treated seeds and baits the recommended design is probably less severe than the 'rigorous' version A of the BBA test, which provides less untreated food but is more severe than the 'normal' version B.

Some degree of exposure by non-dietary routes (inhalation, dermal absorption) is possible in the critical test. If it is thought that exposure by these routes might contribute significantly to the overall risk, then the test design should ideally be modified to ensure that those contributions are realistic, unless they can be taken into account in other ways.

Comments in the following sections are considered relevant to all three categories (non-food, foliage and other food) except where otherwise indicated.

Test subjects

- Ideally, the species most likely to be at risk would be used in the test. However, there will often be a range of species at risk, most of which it would be impractical or unacceptable to test in the laboratory. It would therefore usually be preferable to use a representative surrogate species.
- The species should be ecologically relevant. For example, common species that can acclimate to captivity may be selected to represent corn field birds (quail, ring-necked pheasants, red-winged blackbirds, sparrows).
- The species should be metabolically relevant, i.e. of similar body size and diet to those at risk. Small-bodied species should be preferred for testing granules since, for some chemicals, they may require only a very small number of granules to receive a lethal dose, which restricts their opportunity to develop learned avoidance.
- Body condition should be equivalent to that at the time of year when exposure could be expected. Particular care will be necessary in simulating the body condition of birds during migration, where this is relevant (e.g. in the case of waterfowl on turf).

Accommodation (housing)

- The number of animals per pen should take account of the group size which is ecologically relevant for the test species, the need to measure individual consumption (requiring individual housing), and practical limitations on pen size. The group considered that group sizes of up to 5 might often be used for smaller species, whereas larger species would frequently be housed individually.
- Pen size would be dependent on species, number of animals per cage, the area required for sufficient spreading out of the test material (see later), and animal welfare considerations. In testing the repellency of treated seeds by comparing bird use of treated and untreated plots within a large flight pen, Daneke and Avery (1989) found that larger plots (78 or 108 m²) were more readily discriminated and gave more consistent results.
- In the case of *foliage*, suitable plants could be grown and sprayed in permanent aviaries. In practice, the nutritional value of such foods is too low for adequate supplies to be grown in the space which is usually available. In this case, temporary pens may be constructed on field plots containing growing plants which have been sprayed with the test material.

- The group felt that it would usually be more practical to use outdoor pens, due to the space required. Ambient conditions will influence results, so concurrent control birds will be needed (see later).

Time of year

- The group recognised that seasonal factors could affect bird condition and behaviour, particularly feeding rates. Consequently, it may often be preferable to conduct the tests at the time of year expected in real exposures.

Preparation of animals

- A 5 to 7 day period for acclimation to test conditions, or longer if required to stabilise daily food consumption, was recommended. Longer periods may often be required for birds obtained from the wild.
- Food presented during acclimation may be standard aviary diet. In tests for foliage and other foods, it may be desirable to acclimatise birds to the alternative food to be presented in the treatment phase (see below) if this food is a normal constituent of the diet in the wild. The group discussed the case of *treated seed*. It was felt that those designing tests should consider whether seed presented in the acclimation phase should be dyed (but untreated) if most seed available in the wild was dyed. This might be necessary in some cases to prevent responses to seed colour having more influence in the test than they would in the wild. This is an issue which requires further research.

Test material

- As the emphasis is on realism, the test material in the critical test would usually be the formulation. This implies that separate tests may be required for different formulations, unless there is good reason to believe the results would be equivalent.

Critical Test Procedure

Treatment levels

- Consultation between regulator and registrant is particularly important in deciding the number and setting of treatment levels because of their effect on the cost of the test, the number of animals required, and on the potential to extrapolate results to other concentrations relevant to the risk assessment.
- It is expected that a bird would encounter a range of doses in the wild. This can occur because of patchy spray applications, variability in the formulation process for seeds (e.g. Jahn 1991), granules and baits, and decay of residues over time. The group noted that even if the maximum residue concentration is repellent, lower concentrations may be sufficiently palatable for the animal to ingest a lethal dose. A range of concentrations should therefore be selected to provide a reasonable chance of detecting such a hazard, taking account of the range of residue levels expected in the field. It may be desirable to collect residue data from the field specifically for this purpose if existing data and estimates are inadequate.

- The range of concentrations should include the maximum expected in the field. This may somewhat exceed those predicted for the recommended application rate, to allow for variability in application techniques (this may apply to seed treatments, granules and baits as well as sprays).
- The group considered that up to 4 treatment concentrations might often be appropriate.
- The group briefly considered the possibility of sequential exposure to the same product, possibly at differing concentrations, for example when it is used in several fields in one locality. Research has suggested that, for some compounds, sub-lethal exposure may reduce ability to avoid subsequent exposures (Bussiere et al. 1989). It was suggested that if there were cases where this was a significant concern, treatments might be offered in a 'flip-flop' design, with some birds receiving a high concentration followed by a low concentration and other birds receiving the treatments in the reverse order. However, it would be difficult to determine what combinations would actually be encountered in the wild.

Controls

- There should generally be one untreated control treatment, in which birds are kept under identical conditions to those in other treatments (including diet) except that the test material is omitted. This is required in order to assist in the interpretation of mortality and sub-lethal effects seen in the other treatments, and to determine whether the test conditions are biasing the test by deterring consumption even in the absence of the test material. In the case of *non-food items*, if the items are the formulation (e.g. granules), they should be completely omitted rather than being replaced with untreated items. The same number of replicates should be performed for the control as for other treatments, and the same observations should be made.
- The group discussed the possibility of using positive and negative controls to confirm that the test was capable of correctly distinguishing between chemicals which would be avoided in the wild (e.g. methiocarb) and those which would not (e.g. dieldrin, anticoagulant rodenticides). It was felt that, provided the recommended test design were successfully validated (see later), it would be preferable not to include such controls in every test for reasons of welfare and economy. However, in some cases the test design may have to be modified so much (to represent particular risk scenarios) that it requires additional validation. If negative or positive controls are to be included, their nature should be the subject of detailed discussion between regulator and registrant as they would be crucial to the interpretation of the test results.

Fasting

- Birds should be fasted for an appropriate period every night to simulate normal diurnal restrictions on feeding activity. The feeding period may need to be further restricted to simulate limitations on feeding which wild birds experience during daylight hours, for example as a result of predation risk, human disturbance or environmental factors (e.g. bad weather or snow cover).

- Wild birds usually have higher energy requirements than captive birds and may show higher consumption rates, which would increase the risk of ingesting a lethal dose before developing an avoidance response (Mineau et al. 1994). Artificially shortening the feeding period (e.g. Rogers 1974) may provide a means of increasing consumption rates to simulate such effects.

Duration of treatment and post-treatment periods

- The treatment period should be at least long enough to ensure that, if the test material is not avoided, a lethal quantity will be ingested. This period could be as little as 1 day. However, a treatment period of about 4 days would usually be preferable to allow detection of delayed effects (e.g. due to mode of action or partial avoidance), delayed learning or, in the case of an early response, habituation. Even if treated food in the wild is typically available for only a short period on any one field, it is likely to be available over longer periods on neighbouring fields within the foraging range of individual birds.
- Test diets should be presented for the whole of the feeding period each day. *Non-food items* could be made available continuously.
- Post-treatment period: as in screening test (see earlier).

Early termination of test on welfare grounds

- As in the screening test (see earlier).

Presentation of test material

- For the purpose of realism, the test material should be presented in a way which is representative of what the animal is expected to encounter in the field.
- The group considered that in general the test material should be spread out rather than presented in pots, hoppers or small trays. Limitations on space are likely to constrain the density of the test material to much higher levels than is usual in the field, but it must be low enough to avoid vapour pressures of the test compound significantly higher than those in the field. Avoidance of higher vapour pressure would also be assisted by conducting the test outdoors, or otherwise ensuring high levels of air exchange. A re-analysis of existing data on consumption in LC50 tests, where test diet is presented in pots, shows a significant negative correlation with vapour pressure, suggesting an element of avoidance which is unlikely to occur in the field (unpublished analysis by Hart using data from Hill and Camardese 1986).
- *Foliage* should be presented as growing plants, as discussed earlier.
- Other food types and non-food items should be presented against a background representative of those expected in the field. Often this may be soil of an appropriate type, or turf.

- In the case of non-food items, an appropriate untreated diet should be scattered onto the same surface as the test material. Food and treated material should be spread separately to avoid contamination of the food (BBA 1993). The food should be representative of the birds' diet in the wild. In particular, a seed diet should be provided for granivores as it may increase the use of grit and the release of active substance from pesticide granules in the gizzard.
- In tests of granular pesticides, treated granules should be spread on a soil floor. The density of granules and their incorporation into the soil is a matter requiring careful consideration and agreement between registrant and regulator. For example, as a reasonable worst case the density might be set equivalent to the maximum application rate for field use, assuming no incorporation into the soil. Although this would be higher than the surface densities achieved by many methods of application, it would be lower than occurs in spillages and when turning at row ends. In addition, it may be desirable to use artificially high densities to increase the chance of detecting accidental ingestion of granules (mortality of a few per cent might be considered significant in the wild, but would be unlikely to be detected in a relatively small test group).
- The soil used for the background in tests with *granular pesticides* should also be considered carefully. If it contains large proportions of grit (e.g. sandy soils), this may reduce intentional ingestion of the test granules. Grit content should represent the lower end of that likely to be encountered when the granules are used in the field, such as a clay silt. Bird preferences for different grit types have been the subject of a number of research studies (Best 1992, Best and Gionfriddo 1991, 1994a, 1994b, Gionfriddo and Best 1995).
- Ideally, test materials should be removed and replaced daily to ensure consistency in test conditions between days. Alternatively, birds could be moved to freshly prepared cages or given access to different areas of the same cage on successive days. A possible exception is *granules*, if they are provided in great excess and do not suffer significant decay of the toxic ingredients. To facilitate removal of test materials other than granules, consideration could be given to offering them on large trays or other hard surfaces, provided it is agreed that the decrease in realism is unlikely to affect the result.

Provision of grit

- For species that feed primarily on seed, the subjects may require grit to break down their diet. If none is available, the birds may be unable to maintain normal weights and may not absorb as much of the active ingredient as they would in the wild. In general, therefore, an adequate supply of grit should be provided either in soil or separately (e.g. in pots).
- In tests of grit-like *non-food materials* the only alternative grit should be that available in the soil (see above).

- Birds deprived of a grit source will tend to conserve the grit remaining in their gizzards. If their grit load becomes depleted, however, they will increase their rate of grit consumption when a new source is presented. The group therefore considered whether grit should be withheld for a period prior to tests with *granules* to make these tests more severe. At least it seems prudent to avoid offering an unrealistic excess of grit (e.g. presented in pots) during the pre-treatment period.

Choices

- The group considered this a crucial aspect of test design. The availability of alternative foods varies widely in the wild. There will nearly always be some alternative to contaminated food in the wild, but it may be unpalatable, far away, difficult to find, etc.
- Standard dietary toxicity tests provide no choice, which the group considered unrealistically severe and therefore likely to lead to the potential being significantly underestimated.
- The group regarded simple two-choice designs with large amounts of untreated food as too unconservative for the critical test, because birds may learn to avoid the treated food more easily than in the wild and at little or no cost in time or energy.
- Another concern about simple two-choice tests was that, if the alternatives were presented in two large, separate patches, the birds may make very few active choices at the start of each feeding bout. The group felt it was preferable for alternatives to be presented in mixtures or chequered arrangements, which would require the animal to make more choices, increasing the severity of the test. However, the group recognised that the realism of this is debatable; choices between treated and untreated fields may be more like the simple two-choice test, but variation in application rates within fields may be high enough to resemble the mixture presentation.
- Presentation in mixtures would increase the severity of the test by making it difficult for birds to selectively avoid test materials unless they have significant primary repellency, such as an aversive taste, or at least a distinctive taste which facilitates secondary repellency (conditioned aversion).
- In most cases the presentation of mixtures would make it impractical to measure consumption of treated and untreated material separately (see below).
- Another important consideration is the nature of the untreated alternative, and the quantity provided. The group was concerned that the use of familiar aviary diet as an alternative to treated food of a different type would reduce the severity of the test excessively in some cases. The group considered that in general the alternative in the test should be representative of the choices available in the wild. As stated above, it may be desirable to acclimatise the birds to the alternative food during the acclimation period.

Non-food items

- There is no need to provide untreated granules. Alternative grit would be available in the soil substrate (see above), interspersed with the test granules.

Foliage

- If an untreated alternative is provided, then it and the treated plants would be presented in two or more separate plots within each aviary. A chequered arrangement would increase the frequency of choices made by the birds, but is difficult to arrange. It may be preferable to ensure severity of the test by providing no alternative to the treated foliage. Provision of standard aviary diet in hoppers, etc. as an alternative would probably reduce severity too far, unless the amount offered were substantially less than the normal daily intake. Research is necessary to investigate these alternatives.

Other food

- Treatment groups should receive a mixture that consists of several parts treated material and 1 part alternative food, spread out on the floor. The proportion of treated food should be high to ensure a suitably severe test, and should be at least enough to ensure birds in the highest treatment group will suffer a lethal exposure if they fail to discriminate between the two foods. A ratio of 3 parts treated to 1 part alternative might be appropriate.
- For seeds, insects and fruits, the alternative food could be the same as the test material, but untreated, because this choice will be available in the wild. However, this may be thought to make the test too severe if inability to discriminate between the alternatives causes birds to respond by avoiding eating altogether, since in the wild a wider range of choices would be available. Consideration might therefore be given to including in the mixture a second untreated food, of a type visually distinct from the other two.
- In the case of baits, the alternative could be a standard aviary diet. Care is required in selecting this diet to ensure that its palatability relative to the bait base is representative of choices likely to be available in the wild.
- The alternative food should be offered at an amount below normal daily intake, in order to promote sampling by the bird and increase the severity of the test. The amount should be set to be a little more than enough to ensure that no mortalities through starvation will occur if birds consume only the untreated food for the duration of the test. Some indication of what this amount should be may be available from a dietary toxicity test, if one has been done with similar species and food material, or from food intake during acclimatisation of the test subjects. The amount might be about 75% of normal daily intake (this is less severe than the 'rigorous' version of the BBA test, which limits untreated food to 25% of normal daily intake).
- The number and types of choices offered, and the quantities of each, are critical issues. Research is needed to investigate how these choices should be set in order to ensure that tests are realistically, but not excessively, severe.

Measurements

- Mortality, sub-lethal effects, body weights, environmental conditions, and the residues in the test material should all be recorded as in the screening test. Observations for mortality and sub-lethal effects must be made at the end of each day. Ideally, additional observations should be made during the day to enable prompt removal of subjects on animal welfare grounds should this prove necessary. This requires that means are available for making observations without disturbing the birds, e.g. from a hide or blind. Methods for observations for sub-lethal effects could be guided by data on effects recorded in acute, chronic and reproductive studies if these are available.
- Total daily food consumption should generally be measured during acclimation and pre-treatment periods to assist in deciding quantities to offer during the treatment period (see above).
- Total daily food consumption should be measured in the test period to confirm that subjects are feeding normally under the test conditions and to provide a means of detecting generalised reductions in intake (anorexia). If direct measurement of intake is impracticable, feeding behaviour may be monitored by remote observation or using video-recordings, although the correlation between feeding movements and ingestion may be weak and could differ between treated and untreated groups.
- Daily ingestion of treated and untreated materials in the treatment period should be measured separately if permitted by the manner of presentation, which may require sorting of mixed test materials by hand or other means. This will generally not be practicable for granules, but could be considered for most other types of material (including pellets and pillorized seeds). It may be sufficient to sort a sub-sample from each cage, and to do so only on selected days.
- Otherwise, other methods of assessing relative intake should be considered. Chemical markers (Savarie et al. 1992) might be used to assess the proportion of test material remaining, provided the markers were known not to affect palatability or toxicity. Alternatively, physiological biomarkers (e.g. cholinesterase inhibition for organophosphates) might provide a measure of intake of the test material, if they could be adequately calibrated.

Endpoints

- In this test the primary endpoints are mortality and sub-lethal effects, which are used to assess whether avoidance may contribute to reduction of mortality in the wild. Interpretation of the results will require expert knowledge (see later).
- Measurement of avoidance as reduced intake or body weight would be a secondary endpoint. The most important function of this measurement is to confirm that consumption and body weight are maintained at normal levels by birds in the untreated control.

- If no mortality or sub-lethal effects occur, data on body weights and ingestion may be helpful in assessing whether the reduced risk was due to avoidance rather than some other cause (e.g. if the pesticide is significantly less toxic to the species used in the avoidance test than to species used in standard toxicity tests).

Statistical aspects

- Those conducting tests should obtain expert statistical advice on both experimental design and data analysis. In addition, it would be desirable for regulators and registrants to agree in advance on the role of statistical significance in interpreting the results (see later) and the degree of statistical power required.
- The group was advised that for continuous data (e.g. body weights, consumption) 5-8 replicates (i.e. cages per treatment) would usually be adequate. This number is unlikely to be adequate for testing the statistical significance of differences in frequency data (e.g. mortality, qualitative symptoms of toxicosis) unless the number of animals per cage is substantial (5-10) or the differences are very large.
- As in the screening test, stratification or blocking of subjects should be used if this helps to improve precision and reduce the numbers of animals required.
- Comparisons between formulations (e.g. for development purposes or in comparative risk assessment procedures) would pose different statistical requirements than examining only one formulation.

Interpretation

Screening test

The group viewed the screening test as an optional preliminary step with the primary purpose of determining whether it is worth proceeding to a critical test. The screening test should **not** be used on its own as evidence that avoidance will be effective in the wild, for the reasons given earlier. If the results of the screening test indicate strong avoidance at the concentrations expected in the field, then the registrant is likely to be encouraged to proceed to a critical test. Comparison with published values for known repellents such as methiocarb may be helpful in reaching a decision, though care is required when comparing-tests conducted with different species.

If the screening test provides no evidence of substantial avoidance, or if it results in mortalities at concentrations equivalent to those expected in the wild, then no critical tests would be conducted. Avoidance will be disregarded in further refinement of the risk assessment unless there is compelling evidence from other sources such as field studies.

Data from the screening test may also be useful in refining the interpretation of the critical test (see below).

Critical test

The group's view of the implications of different test outcomes for the primary endpoint is summarised in **Table 5.3**

Table 5.3 Implications of different outcomes of the critical test

Result	Probable implication
Toxicant-induced mortalities	Avoidance does not adequately reduce risk and should not be a factor in refining risk assessment
Adverse sub-lethal effects	Avoidance may partially reduce risk: options - <ul style="list-style-type: none">• confirm using field studies• apply risk mitigation and retest
No adverse effects	Avoidance is probably a factor in refining risk assessment: need to ensure through post-registration monitoring

It is essential to have some way of reliably distinguishing toxicant-induced mortalities from any background mortality which might occur in the test conditions (though this should be negligible if healthy birds are used). Where possible, the cause of death should be determined by appropriate post-mortem investigations. If background mortality cannot be eliminated and cause of death cannot be reliably determined, then the decision will have to be based on a statistical comparison of mortality in treated and control groups. This should be avoided, as it is likely to greatly increase the number of animals required.

Avoidance may remain a factor in the risk assessment despite mortalities in the critical test if the frequency of mortality is low, and if there is direct evidence (e.g. from consumption data) that it has been reduced by partial avoidance. In this case, the action would be as shown for adverse sub-lethal effects in Table 5.3.

Consideration should be given to whether sub-lethal effects are adverse (toxicosis) or the consequences of potentially adaptive responses (e.g. reduced food intake and associated loss of body weight). Results on consumption in the screening test may assist in making this judgement. The severity of adverse effects could be taken into account in two ways: would they be unacceptable in themselves? and what do they imply regarding the extent to which the risk of mortality is reduced by avoidance? Adaptive effects and adverse effects judged to be minor would be treated as 'no effects' in the table above.

Evidence on the mechanism of avoidance (e.g. from the screening test) may be taken into account, but requires particular care and expert interpretation. Avoidance may be caused by primary repellency (reaction to sensory stimuli such as bad taste or smell) or a learned aversion which develops after post-ingestional illness. The first may be overcome by habituation or hunger, whereas the latter may not have time to develop if the speed of ingestion is high (e.g. due to hunger) or the source of the toxicant cannot be distinguished. More weight might be placed on avoidance for chemicals shown to operate through both mechanisms (at realistic exposure levels). It is essential that consideration is given to whether it might be possible in some circumstances for rapidly-feeding birds to ingest a lethal dose before developing an avoidance response. Generally this should be considered in advance and should influence the design of the critical test. If the possibility of such an effect is recognised after avoidance testing, despite a 'no effects' or 'sub-lethal

effects' test outcome, the regulator would be justified in asking for further tests with the relevant species and conditions. Further research is required to identify the circumstances under which such effects may occur.

Those interpreting the results of the critical test should be alert to the possibility that exposure may be due in part to non-dietary routes (inhalation, dermal absorption), as noted earlier.

In the two categories of outcome where avoidance is considered to reduce risk at least partially, further action is indicated in **Table 5.3**. The group considered that this is likely to be required because of the lack of certainty about the extent of risk reduction and about its applicability to the wider range of species and environmental conditions in the field. **Risk mitigation** would involve finding ways of modifying the formulation or manner of use of the pesticide so as to enhance or supplement the partial avoidance and to reduce risk to an acceptable level. The effectiveness of such methods would need to be confirmed, probably by subjecting them to further realistic tests similar to the critical test. **Field studies** would be experimental studies aimed either at demonstrating avoidance directly in the field, or at confirming that the level of mortality in the field is acceptable and consistent with reduced risk. It should be noted that the design and interpretation of field studies is difficult and the subject of some controversy (Somerville and Walker 1990). **Post-registration monitoring** would be carried out for a suitable period, during which sales of the material might be limited and after which the registration would be reviewed (if allowed by the relevant regulations). Monitoring should be targeted (e.g. based on visits by company staff to selected sites where the product is used) unless a routine system of monitoring exists which would be sufficiently sensitive to detect effects if they occurred.

Limits to Extrapolation

The critical test should be designed to represent a specific use scenario, usually one which led the regulator to consider that a high risk might occur. It might be desirable to extrapolate the result to a range of other conditions, for example:

- (1) other avian species;
- (2) other concentrations of active ingredient in test substrate;
- (3) other substrates (including new formulations, where substrate is a formulation);
- (4) other crops;
- (5) other environmental conditions.

Type (1) above is nearly always necessary to some extent. Protection against unexpectedly large species differences may be provided to some extent by requirements for field tests or post-registration monitoring, as discussed above. Type (2) is also often necessary. It may be achieved by interpolation between the treatment levels in the critical test (provided they are not too widely separated), or by considering the results of the critical test in conjunction with results of the screening test (if this was done, and included a wider range of concentrations). Types (3) and (4) might be desirable to assess a change in formulation or use proposed by the registrant subsequent to the original test being performed. In such cases the regulator would be justified in requesting a new test if the new use or formulation were sufficiently different to make extrapolation unsound. Type (5) should be considered at the outset. If the range of conditions is too wide to be represented by a single test, then more should be done.

In deciding on the number of tests required, both regulator and registrant should be conscious of the need to avoid unnecessary testing for both economic and animal welfare reasons. It will be preferable for the registrant to carry out tests one at a time and consult the regulator after each one, as the results may indicate that the subsequent tests are no longer necessary.

Protection Against False Positives

A false positive would occur if the test outcome indicated that avoidance would reduce risk when, in the field, it did not. Aspects of the Working Group's proposals which contribute to protecting against a false positive are:

- realism of test species and conditions;
- some test conditions tending to worst case, e.g. highest treatment level, density of exposed granules;
- subjects in nutritional condition representative of field scenario;
- pre-fasting overnight;
- continuous simultaneous choice (mixed treated and untreated food);
- pre-conditioning to test substrate without active ingredient, where this is realistic;
- special consideration of the possibility of unusually rapid feeding;
- use of the untreated control treatment to check for spurious reductions in consumption;
- validation of the test method with a range of repellent and non-repellent pesticides (see below).

No-choice tests would probably be more conservative in most cases, but were felt by the group to be less realistic. The group recognised that, despite the precautions listed above, it would not be prudent to rely solely on any test of avoidance using captive birds, no matter how conservative. It was for this reason that the group placed great importance on the use of post-registration monitoring to detect false positives and provide the opportunity for corrective action. When false positives are detected, their causes should be carefully investigated to decide whether changes in test design are necessary.

Animal Welfare

- The group considered the welfare of test subjects at several points, including the choice of species, group size and pen size, degree of replication, and early termination of the test as a whole or for individual subjects (see above).
- The group recognised that for primary (but not secondary) repellents an assessment of avoidance could be based on reductions in consumption alone, without allowing substantial intoxication, but considered that this would be unconservative and could result in the regulatory process failing to prevent mortalities occurring in the wild.
- Those designing individual tests should seek to improve the welfare of subjects as far as possible without prejudicing the essential function of the test.

- Testing should be avoided wherever extrapolation provides a reliable alternative.

Recommendations for Further Work

The uncertainty which remains about the reliability of tests with captive birds in predicting avoidance in the wild makes it essential to validate the proposed methods for a range of pesticides, including those thought to have given misleading results with other tests (e.g. diazinon) as well as some usually regarded as repellent (e.g. methiocarb) and some which are not repellent (e.g. dieldrin, anticoagulant rodenticides). In addition, many aspects of the methodology proposed by the group require refinement. In the interests of harmonisation, attention should be focused on the extent to which test design may be standardised.

Methodology

1. Experiments to confirm whether presenting the test material in pots or hoppers is adequate, or whether more realistic methods are necessary, should be considered.
2. Circumstances under which repellency fails to protect against intoxication (e.g. when the ingestion rate is high) should be investigated, and ways of incorporating these circumstances in test design should be developed.
3. The contribution which repeated exposure to the same product makes to risk in the wild should be assessed and, if it is significant, consideration should be given to how this could be included in test methodology.
4. The less well established aspects of the proposed methodology, including pen trials and ways of deciding the types and quantities of treated and untreated foods to be offered (in both the acclimation and treatment phases), should be refined.
5. In the light of the above, it should be determined whether more specific guidelines should be drafted for particular types of avoidance test (e.g. for seed treatments).

Validation

6. Existing data from dietary studies, palatability studies, field studies and poisoning incidents should be reviewed to assess the reliability of laboratory-to-field extrapolation of avoidance (this might be done by an OECD/SETAC working group, to relieve concerns about commercial confidentiality).
7. The recommended test design should be conducted for a selection of existing pesticides and the results compared to (a) existing avoidance data from other tests, and (b) field studies and incident data.
8. In the light of the above, the test design should be reviewed and modified to remove unnecessary aspects, in order to improve cost-effectiveness and minimise the suffering of test subjects.

Supporting actions

9. As explained above, it is necessary to place heavy reliance on post-registration monitoring to detect cases where avoidance fails to prevent mortality in the wild. Moreover, field studies are proposed as one option in cases where adverse sub-lethal effects occur in the critical test. There is a need to consider whether existing approaches are adequate for these purposes or whether further development is required.
10. A task group should be formed to co-ordinate actions 1-9 and to refine the guidance document or develop guideline(s), as appropriate.

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APPENDIX

Experience with Acceptance Tests with Birds According to BBA-guideline VI 25-1

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The repellency test according to BBA-guideline VI 25-1 was introduced in the German authorisation procedure in the early 1980s. Since then, 42 studies have been submitted. The number is relatively low, because the test is conducted only in the case of toxic granules, seeds and baits. Unfortunately the results of the studies are confidential according to present legal regulations in Germany, and therefore can only be presented in an anonymous form.

The results are presented in tabular form **on the next page**. Often more than one study was submitted for a certain product (different species or different types of seeds); Such studies are combined in the table. Some of the products did not gain authorisation in Germany (due to different reasons).

ai: The active ingredients (or combinations) are numbers; a certain ai may appear in two categories.

Mortality: In test A the test substance and standard diet are offered in a ratio of 3:1; in test B the ratio is 1:9. If mortalities occur in both tests, the risk is considered as high; if mortalities only occur in test A, the risk is considered as medium. However, this is only a crude interpretation because the actual risk in the field depends on dosage (items per area) and technique (incorporation or surface application).

Incidents reported in Germany (Joermann and Gemmeke 1994): The entries in the table are related to the product in question. However, the reporting system is not well developed in Germany, so the absence of incidents is not proof of safety. Some products are new on the market or used on a small scale, so that incidents would not be expected.

Incidents reported in other countries: Information is derived, for instance, from the UK (e.g. Fletcher et al. 1994) and the USA (e.g. Smith 1987). The cases refer to the same type of formulation, but not necessarily to the same product.

Table 5A1: Comparison of the results of BBA acceptance tests and data from post-registration surveillance

Type of formulation	ai	Mortality:		Authorisation in Germany	Incidents:	
		Test A	Test B		Germany	Abroad
Granules	1	+	+	+	+	+
	2	+	+	-		-
	3	+	+	-		-
	4	+	+	+	-	-
	5	-		-		-
	6	-		-		-
	7	-		+	-	-
Seeds	1	+	+	+	+	-
	8	+	+	+	+	-
	9	+	+	+	-	-
	10	+	-	+	+	-
	3	+	-	-		-
	11	+	-	+	- ¹	-
	12	-		+	-	-
	13	-		+	-	-
Baits	14	+	+	+	+	+
	15	+	+	+	- ¹	-
	16	+	-	+	-	+
	12	+	-	+	-	+
	17	-		+	- ¹	-

¹ incidents hardly to be expected (new products, small scale of use)

6. Framework for the Use of Avian Tests in the Risk Assessment of Pesticides

Introduction

As well as producing guidance on the design and practical aspects of avian testing, the Workshop's objectives included the development of proposals for a framework to govern the way these tests are requested and used in practice in risk assessment. The focus for development of this framework was principally in relation to predictive risk assessment for classification, registration and approval for use of pesticides, because the majority of avian testing is done in these contexts.

This chapter focuses on the role in environmental risk assessment of the tests recommended by the four Working Groups (i.e. Acute Toxicity, Dietary, Reproduction and Avoidance). There is first a brief outline of the general principles that underlie the approaches used by regulators to predict risks arising from pesticide use. This outline does not attempt to reflect the particular procedures of individual regulatory agencies, which differ in many ways. Rather, it concentrates on the basic strategies followed, which are common to most schemes. The set of tests proposed as a result of the Workshop is then reviewed, and the various ways they can be combined within risk assessment schemes are discussed.

It must be noted that, due to time constraints at the Workshop, this 'framework' was developed largely after the meeting by the Workshop Chair in consultation with the organising committee. Workshop participants were given the opportunity to comment on the proposal when the draft Workshop report was circulated for review in November 1995. This version incorporates the comments received.

Principles of Ecological Risk Assessment for Birds

Risk assessment for effects on birds is based on the same principles that are used for all ecological risk assessments, i.e. it involves a comparison of the ratio between estimated toxicity and estimated exposure in order to predict risk. There must be a balance between the need to avoid false negative outcomes as far as possible (i.e. not judging harmful products as safe), and the dangers of being overly precautionary.

In the transition from basic information about a chemical and its proposed pattern of use to the classification of expected risks to birds, laboratory avian tests may be needed at three stages :

- preliminary screening to determine the existence of a potential toxic hazard;
- simple assessment of the likely scale of the hazard compared to other products or to a threshold criterion, based on a ratio between initial estimates of toxicity and exposure;
- refinement of the assessment by reducing uncertainty in either toxicity or exposure estimates, or both.

There are a variety of possible assessment strategies in which avian testing may play a part, depending on the chemical and its use pattern, the bird species at risk, and the aims of the assessment.

Overall Strategic Approaches

The need for a particular kind of test at a particular point in risk assessment is governed by the overall goals of the assessment. In a particular case, the assessment will be driven by one or more of the following seven major objectives. As a result, the nature of the testing required (i.e. which tests are requested, and how they are designed) will vary from case to case.

These objectives are not listed in order of importance. Indeed, the weighting given to each of them will differ among the parties involved in assessments. It will also be clear that some of them appear to conflict, so that it is not possible to address all objectives fully by a single strategy. The art of ecological risk assessment lies in the ability to strike an acceptable balance between conflicting priorities.

Objective 1: To generate data which will cover risk assessment of all proposed or potential uses of an active ingredient in pesticide formulations.

This implies:

- (i) It is more appropriate to base test doses on the range necessary to provide a proper dose-response relationship than on expected environmental concentrations. In this way, the test will generate data relevant to possible future uses of the chemical at higher dose rates, without the need for further testing.
- (ii) Testing should use active ingredients, not formulations.
- (iii) Tests should cover all forms of exposure (from acute to chronic time scales, and dietary presentation as well as oral dosing), regardless of likely exposure in the immediate case under assessment.
- (iv) It might be appropriate to emphasise the mode of action of the compound, rather than routes of exposure, when choosing the endpoints to be recorded.

Objective 2: To produce the most relevant data for making a detailed and specific risk assessment of a particular pesticide use.

This implies:

- (i) Dose rates used in tests should be chosen according to the levels expected to occur in the field, although they should incorporate a safety margin to allow for the variability of environmental concentrations.
- (ii) Other aspects of test design should also be modified according to knowledge about likely exposure arising from the proposed use. For example, the fate of

residues will dictate the time periods of exposure that are appropriate to simulate in the laboratory, and the relevant exposure routes.

- (iii) It is appropriate to take account of factors that may influence ingestion in the field, such as avoidance of a repellent chemical.
- (iv) When it is likely that the toxicity of the active ingredient is different from that of its formulations, it may be desirable to use the formulated product in certain kinds of tests. This will generally apply at later tiers of testing, rather than in the early stages.

Objective 3: To provide information that will contribute to standard databases, and thus allow for comparative analyses.

This implies:

- (i) Test methods should be standardised as far as possible, particularly with respect to the choice of species, features of the dosing/feeding schedules, and the endpoints measured.
- (ii) There are advantages in carrying out core tests on a routine basis, so as to provide data that are valuable in the longer term, although these data may not be essential for immediate risk assessment purposes.

Objective 4: To allow conclusions about hazards and risks to be extrapolated from the species tested to all types of birds.

This implies:

- (i) Tests should include species, ages and sexes of birds that allow the use of extrapolation models based on established patterns of species sensitivity.
- (ii) There are advantages in recognising that different factors may influence birds with different ecological habits, and in incorporating this explicitly into the risk assessment (e.g. by conducting parallel assessments for relevant types of birds).
- (iii) Testing should cover a broad range of environmental concentrations, rather than focusing on a particular exposure pattern that is relevant to a few species only.

Objective 5: To produce a precise estimate of risk, rather than assign uses of chemicals to simple broad categories such as 'high' or 'low' risk.

This implies:

- (i) Where possible, tests should be designed to quantify toxicity, not provide 'less than' values.

- (ii) When further testing is carried out to refine toxicity estimates, it is also necessary to improve estimates of exposure, so as to minimise uncertainty in the risk quotient.

Objective 6: To standardise testing as much as possible, in order to promote international harmonisation and mutual acceptance of data.

This implies:

- (i) Test methods should be uniform, even where a test may be applied at different stages of the risk assessment process for different chemicals.

In addition to these direct objectives, there is also a general need to include an additional objective which cuts across all the other priorities:

Objective 7: To minimise the amount of testing carried out (for animal welfare and/or economic reasons).

This implies:

- (i) The design of tests should be based on the minimum numbers of animals and lowest dose ranges that are compatible with a rigorous statistical model for interpretation of the data.
- (ii) Tests should be carried out only when they are needed within a risk assessment, rather than being undertaken routinely in all circumstances. This suggests that attention should be paid to the triggering of tests within a progression of tiers; that exposure should dictate the types of tests required; and that there is scope for omitting lower tier tests when a higher tier alternative subsumes the requirement.
- (iii) Where feasible, use of sub-lethal endpoints that provide an 'early warning' of toxic effects should be encouraged, so as to allow early termination of studies and the use of a lower range of doses.
- (iv) Provided that it is compatible with the use of the risk assessment outcome, testing should be limited to the level necessary to assign uses of chemicals to broad categories such as 'low' or 'high' risk.

Potential Flexibility

Although there are good reasons why risk assessors often do not take a flexible approach in risk assessment (e.g. so as to promote harmonisation, and to provide clear data requirements for registrants), equally there are circumstances in which flexibility is appropriate. For example, the more complex types of tests required at higher tiers cannot be standardised without sacrificing the power to detect and measure relevant effects. There is therefore a need to build in options for 'customising' the design and conduct of some (though not all) tests, often with the aid of expert judgement.

For those cases in which such flexibility is appropriate, there are two types of flexibility to consider — the choice of which tests to carry out, and issues concerning the detailed design of tests. The following possibilities exist:

- (1) An initial judgement of whether exposure of birds is possible provides an opportunity to restrict assessment to cases where exposure is expected.
- (2) Initial information about use patterns can indicate which testing options are suitable, in terms of:
 - the species or types of birds at risk, and hence the choice of test species;
 - likely routes of exposure in the light of the use pattern and environmental fate of the compound, and hence the choice of dosing methods;
 - the time-course of exposure that is expected on the basis of persistence, and hence the need for chronic or acute tests;
 - the mode of action of the chemical, as indicated by physico-chemical properties and baseline mammalian toxicity data, and hence the choice of endpoints for tests.
- (3) Refinement of initial estimates of toxicity, involving one or more of the following aims:
 - greater precision, generally requiring modifications to numbers of dose levels, replication, etc.
 - more realistic conditions, including light and temperature regimes, presentation of food, the character of the diet, etc.;
 - examination of additional endpoints;
 - testing of additional species.
- (4) Refinement of initial estimates of exposure, which will generally involve better fate modelling, direct measurements of residues, etc. but may also include assessment of the potential for food avoidance mechanisms which may affect the intake of the chemical by birds.

Role of Individual Tests

Each of the Working Groups generated proposals for one or more tests within its area, basing the design on each test's intended role within a risk assessment framework. These tests represent the 'building blocks' to be used in answering questions in risk assessment.

Overall, ten tests were recommended (**Table 6.1**). Tests 2 and 3 are substantial revisions of existing guidelines. Test 1 is less radically modified (the test design is little changed, but its use has been reconsidered). Tests 4 to 8 are proposals for new guidelines or less specific guidance. Tests 9 and 10 are recommendations for further areas of testing which should be developed in the future.

Table 6.1 - Recommended suite of avian tests.

Revision/modification of existing guidelines:

- Test 1 - Definitive LD50 test
- Test 2 - Definitive dietary test
- Test 3 - Basic reproduction test

New guidelines or guidance documents:

- Test 4 - Limit test for acute oral toxicity
- Test 5 - Up-and-Down test for acute oral toxicity
- Test 6 - Dietary range-finding test
- Test 7 - Screening test for avoidance
- Test 8 - Critical avoidance test

Tests for future development:

- Test 9 - Short-exposure reproduction test
- Test 10 - Full breeding cycle reproduction test

In the following sections, the suite of tests recommended by the Working Groups are summarised in a common format, using the following six key features:

Role of the test - defined by the exposure period (acute, sub-chronic, chronic), endpoints of concern and route of exposure.

Question(s) addressed - the tiers of risk assessment to which the test applies; the need to identify a potential toxic mechanism; quantification of hazard; etc.

Triggers for the test - properties of the chemical, its use pattern, or results from other studies which would indicate the need to carry out the test.

Flexibility - the importance of standardisation of test design. If there are reasons to have flexibility, what features might be customised, according to the species at risk, the nature of the chemical, and the pattern of environmental availability?

Interpretation of results - use of results in relation to the test's purpose in the risk assessment context.

Triggers for further studies - what further tests might have to be carried out as a consequence of the results of this test?

Test 1: Definitive LD50 Test

Role of the test

Acute oral dosing, primarily for the determination of a dose-response relationship and estimation of the median lethal dose, although other endpoints may be appropriate in special cases when the mode of action is known and there are specific sub-lethal effects

relevant to risk assessment (e.g. behaviour, food and water consumption, sensory functions, physiological effects).

Questions addressed

The definitive LD50 test provides a standard index of inherent toxicity which is used in risk quotient calculations and which is useful for comparative analysis. It can also provide a guide for range-finding tests, and may be used in contexts other than risk assessment, such as establishment of toxic categories for labelling.

Triggers for the test

Triggers are: (1) when there is potential exposure; (2) an effect observed in a limit test for acute toxicity (Test 4). The number of species to be tested depends on knowledge of the test substance: 1 species when the active ingredient is in a well known chemical class; 2 species when the active ingredient is new or little known.

Flexibility

The design of the test and the test species should be highly standardised to maximise comparability. However, there may be scope to add measurements of sub-lethal endpoints without compromising its primary purpose.

Interpretation

The result (i.e. LD50) should be used as the index of toxicity in risk quotient calculations at the initial (Tier 1) stage and may also be used in refined assessment in higher tiers.

Triggers for further studies

The results of preliminary risk quotient calculations may lead to (1) further definitive acute oral tests, if the time taken for a bird feeding on contaminated food to reach the LD50 is one day or less; or (2) a sub-chronic dietary toxicity test (Test 2), if this period is longer than one day. If the risk quotient does not provide adequate confidence, then additional species may be tested by up-and-down tests (Test 5), the number depending on the degree of uncertainty that can be tolerated.

Test 2: Definitive Dietary Test

Role of the test

The test covers exposure through the diet, for periods of up to a few weeks ('subacute' to 'sub-chronic' exposure), to determine the median lethal concentration in food and to quantify other, sub-lethal effects.

Question addressed

The definitive dietary test would not be required for all compounds. The test should be directed at those compounds which are either inherently toxic (indicated by acute oral dosing) or bioaccumulative (indicated by mammalian studies or partition coefficients), or which give rise to cumulative injury in other ways (e.g. anticoagulation). It is used as a

higher tier study, although in view of the fact that there is always likely to be short-term exposure from pesticides, there may be value in carrying it out early in the risk assessment.

Triggers for the test

The test would be triggered by results from the dietary range-finding test (Test 6) showing that the median lethal concentration is less than 5000 ppm in food.

Flexibility

The test would be largely standardised, although there are options to vary the number of species tested (depending on how many LD50 determinations are available) and the sub-lethal endpoints to be included. Treatment levels depend on the results of the range-finding test. It may also be appropriate to add extra levels, in order to many early deaths from the higher food concentrations.

Interpretation

Results on mortality (LC50 determination) will be used in risk quotient calculations, and should be based on exposures calculated from measured food intakes rather than concentrations in food. Attention should be given to the timing of mortality within the test, compared to the likely exposure period. Sub-lethal effects in the absence of mortality may also be taken into account in evaluating risk. The test can provide information on clinical chemistry, pathology, etc. relevant to reproduction tests.

Triggers for further studies

Severe food avoidance in this test may lead to an avoidance test (Test 8).

Test 3: Basic Reproduction Test

Role of the test

The test involves the sub-chronic exposure of breeding birds to determine effects on reproductive performance, particularly the production of viable young, but also including intermediate parameters such as fertility and the hatchability of eggs.

Question addressed

The basic reproduction test will enable the identification of the potential for adverse reproductive effects at exposure levels lower than those which cause serious parental toxicity. The test should be carried out in the first tier of assessment.

Triggers for the test

It should be carried out for all pesticides.

Flexibility

The test design and procedures should be highly standardised, in order to maximise comparability and the statistical power of the test. For first tier testing, a single standard species is recommended, but others may be added at higher tiers.

Interpretation

The main result from the test would be a NOEC — the dietary concentration that shows no reduction in the production of viable chicks. This should be used in risk quotient calculations, along with an appropriate safety factor. However, other endpoints may also be taken into account on a case-by-case basis in deciding whether critical reproductive effects occur independently of toxicity to adult birds.

Triggers for further studies

If there are reproductive effects at a level below 1000 ppm in diet or 2 x PEC (whichever is the higher), no further testing is needed. A safety factor should be assigned to the NOEC to account for interspecies differences. If there are no effects, then the preferred procedure is for an additional test to be conducted on a second species. Alternatively, a very large safety factor should be applied to the highest level at which the first species was tested.

Test 4: Limit Test for Acute Toxicity

Role of the test

The test involves acute oral dosing with mortality as the principal endpoint. Other endpoints could be included if appropriate (as for Test 1).

Question addressed

The test should be used at the initial stage of assessment, in order to assess the need for a definitive acute toxicity test.

Triggers for the test

To be carried out if there is potential exposure of birds but the compound is not expected to be toxic to birds (e.g. based on knowledge of other compounds in the same chemical class).

Flexibility

The test should be standardised in the same respects as the full definitive LD50 test (Test 1), except that the single dose level used is related to the proposed application rate of the compound (but will not exceed 2000 mg/kg).

Interpretation

The results should be used as a preliminary screening for toxic potential before either (a) concluding that there is no toxicity to birds, if the limit test fails to show mortality, or (b) indicating a need to examine acute toxicity further.

Triggers for further studies

When an effect is observed, it would be necessary to conduct one or more definitive acute oral tests (Test 1).

Test 5: Up-and-Down Test for Acute Oral Toxicity

Role of the test

The Up-and-Down test involves acute oral toxicity, and is aimed at identifying an approximate median lethal dose (ALD50), although other endpoints could be included if appropriate (as for Test 1).

Question addressed

The test is a means of reducing uncertainty to an acceptable level. Thus, more species would have to be tested for a more toxic substance because less uncertainty can be tolerated than in the case of a low toxicity substance.

Triggers for the test

If the results of full definitive LD50 tests provide inadequate certainty, a series of ALD50 tests would be conducted on different species.

Flexibility

The choice of species should be flexible, to allow for the availability and logistics of maintenance of different species in different countries. Dose levels would not be standardised.

Interpretation

The results of all the LD50 and ALD50 tests conducted should provide adequate certainty of acute toxicity for incorporation in risk quotient calculations.

Triggers for further tests

There would be no direct requirements for further laboratory testing from the results of the ALD50 tests alone.

Test 6: Dietary Range-Finding Test

Role of the test

The dietary range-finding test deals with exposure through the diet over periods up to a few weeks, as a preliminary to the definitive dietary test (Test 2).

Question addressed

In cases where there is acute toxicity, what are the relevant food concentrations to use for the determination of a median lethal concentration in the definitive dietary test?

Triggers for the test

The test should be carried out if there is evidence of acute toxicity (LD50 below 2000 mg/kg) or potential for bioaccumulation or other indications of possible cumulative damage, or when it takes more than one day to ingest a lethal dose.

Flexibility

Conditions should be the same as in the definitive dietary test (Test 2).

Interpretation

The result should be used solely to indicate the need for a definitive test, and to indicate the appropriate concentration levels.

Triggers for further tests

If the LC50 is less than 5000 mg/kg in food, a definitive dietary test should be carried out.

Test 7: Screening Test for Avoidance

Role of the test

Optional tests involving a simple feeding choice under standard laboratory conditions, to determine the inherent capability of birds to avoid a substance by quantifying reductions in the intake of treated food.

Question addressed

In circumstances where risk assessment suggests that use of a pesticide poses a high risk to birds, the test would indicate whether there is any value in proceeding with a critical avoidance test (Test 8) so as to possibly reduce the estimated expected risk.

Triggers for the test

When risk assessment results in a high risk category, and other information (e.g. evidence of avoidance for related chemicals) suggests that the chemical might have properties that reduce risk in practice. If there are indications of avoidance in a dietary study (Test 2), it may be more economical to proceed to Test 8.

Flexibility

The test procedure should be standardised, with the exception of aspects of design which depend on the species tested (which should be chosen to represent those likely to be exposed in the field, if possible).

Interpretation

The dose level(s) that produce avoidance should be compared against the level(s) that cause toxic effects, in order to determine whether there is any possibility that avoidance can reduce the risk of exposure to hazardous concentrations. In addition, the results may help to clarify the results of earlier dietary LC50 tests in which toxicity appears to have been confounded with avoidance.

Triggers for further studies

If the test shows evidence of avoidance, a critical avoidance test (Test 8) may be carried out; if not, no further attention is paid to reduction of exposure through avoidance.

Test 8: Critical Avoidance Test

Role of the test

A feeding choice study designed to determine whether food avoidance reduces risk in conditions that simulate the field exposure scenario as closely as possible. The primary endpoints are related to possible adverse effects, rather than food consumption.

Question addressed

The test would follow up evidence from the screening avoidance test (Test 7), the dietary test (Test 2) or other sources that there may be inherent potential for avoidance of the chemical, and would seek to confirm or refute that possibility under conditions that more closely resemble the actual exposure situation.

Triggers for the test

An assessment of high risk plus evidence from the screening test, dietary test or other sources, showing that there is a capability for avoidance at levels of intake below those which cause critical toxic effects.

Flexibility

The conditions, methods and procedures should all be highly flexible to enable simulations of particular scenarios, providing assurance that the results will be representative of birds' responses in the wild.

Interpretation

The endpoints are related to toxic effects, in order to obtain a direct indication that avoidance does or does not reduce risk in relevant conditions. If there is mortality, avoidance should not be considered a factor in classifying risk. If there is no mortality, or if there are only sub-lethal effects, it is possible that avoidance may mitigate risk.

Triggers for further studies

If results show sub-lethal effects, the margin of safety may be small, so there should be a field test to confirm the outcome. If there are no effects, post-registration monitoring for safety to birds should be carried out as a check on the effectiveness of avoidance in practice.

Test 9: Short-exposure Reproduction Test

In order to allow for the fact that some pesticide applications result in exposures for short periods that may have substantial sub-lethal effects, an additional test was proposed. This would compensate for the fact that it is not practicable to maintain high exposure

levels for prolonged periods in the basic reproduction test. The test would be at a later tier than the basic test, and would not replace it. Its role would be to reveal any effects on reproduction due to higher doses than are achieved in the longer test, and in some cases it might demonstrate that certain effects emerging after several weeks' exposure are not relevant in a field situation. The need for the test would therefore be triggered by estimates of actual exposure.

Test 10: Full Breeding Cycle Reproduction Test

Some ecologically important endpoints are not covered by the basic reproduction test (for example, the onset of laying, parental competence in incubation, and feeding of young birds). These aspects can only be addressed by developing a test which allows a full breeding cycle to be carried out in controlled captive conditions, and which may therefore be particularly suitable for passerine species. The test would be intended as a higher tier study to clarify the potential for hazard indicated by the basic reproduction test.

Options for Combinations of Tests

There are many ways in which these tests can be combined within practical risk assessment schemes and it is not realistic to define a single 'ideal' scheme that would be appropriate for all purposes. Indeed, it may not be possible to address all the objectives identified in Section 3 (Testing for Dietary Toxicity) in a single approach. Different regulatory agencies adopt different philosophies according to their needs and legal constraints. Therefore, Figures 6.1 to 6.5 are intended to show only how testing can be carried out to investigate each of the various aspects of avian toxicity at different stages of a risk assessment. They do NOT try to illustrate the full sequence of steps that would be involved in an actual assessment, leading to a classification of the expected risk to birds.

Figure 6.1

FIRST TIER - ACUTE TOXICITY

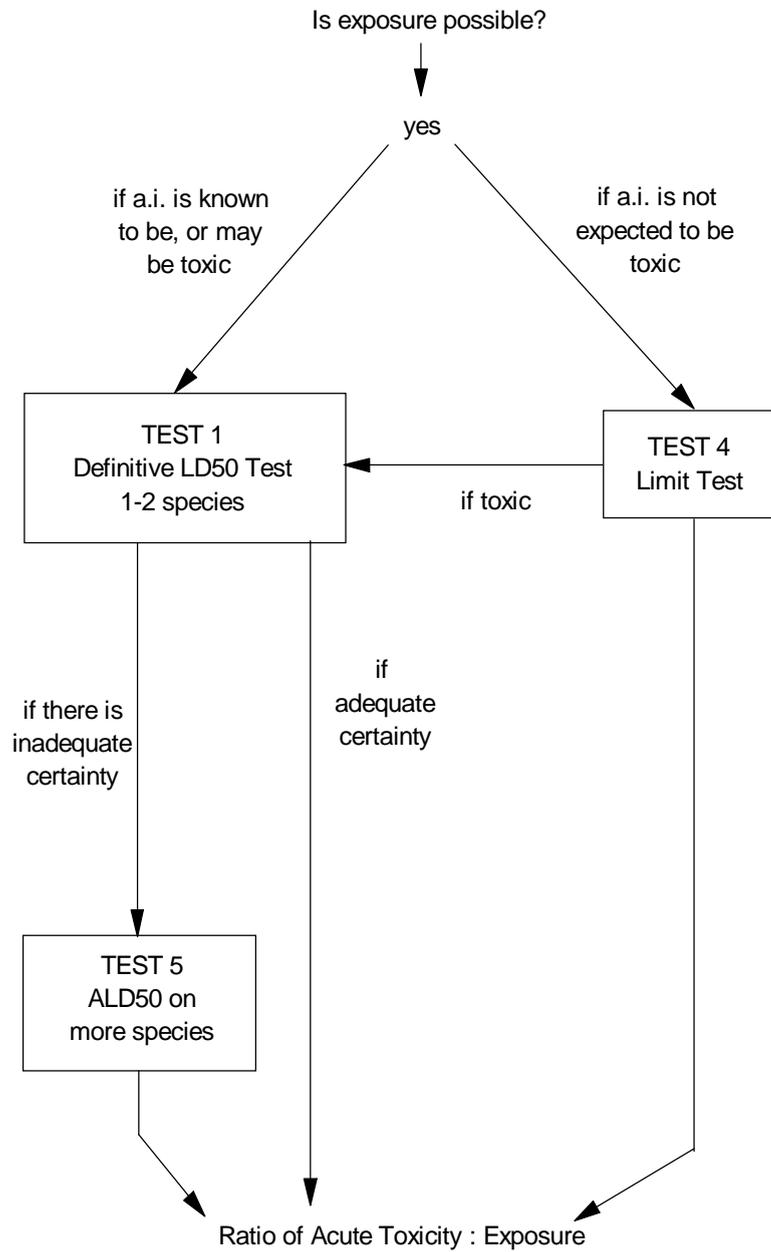


Figure 6.2

FIRST TIER - REPRODUCTIVE TOXICITY

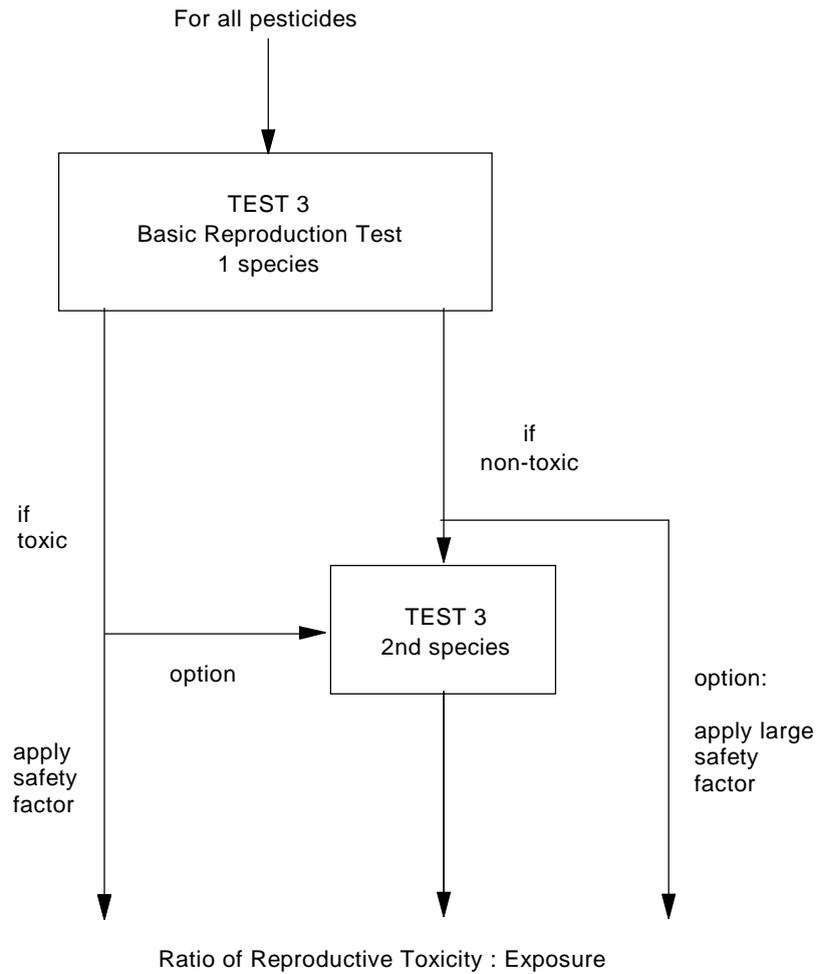


Figure 6.3

LATER TIERS - ACUTE/SUBACUTE TOXICITY

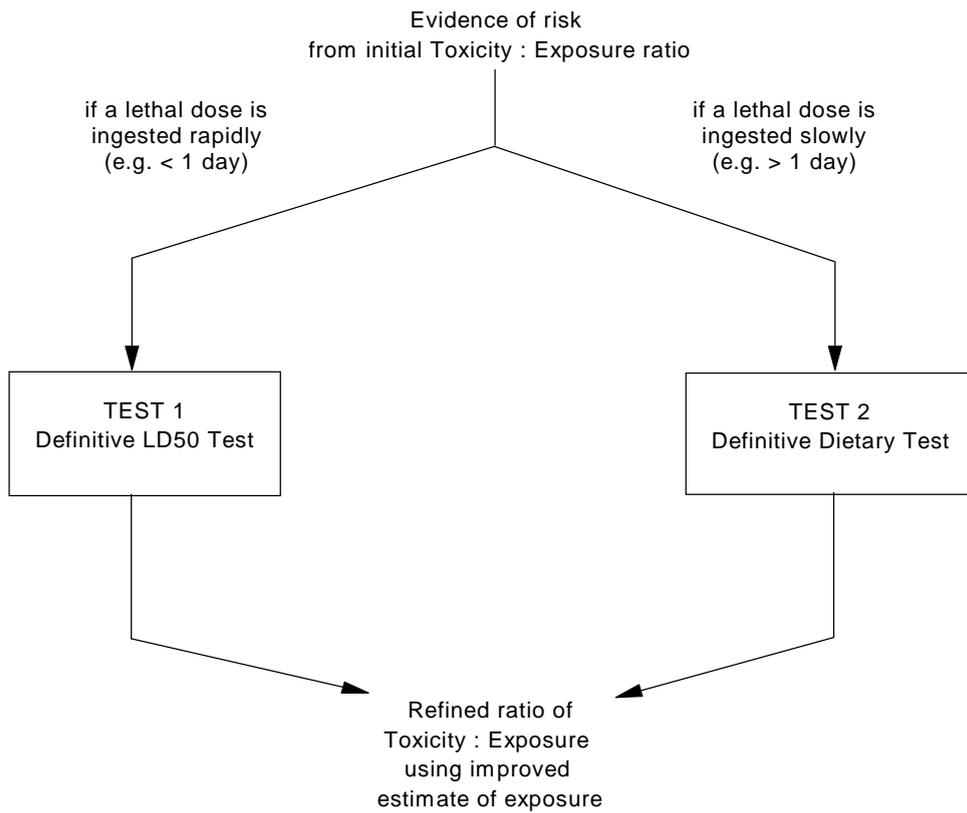


Figure 6.4

LATER TIERS - SUBCHRONIC DIETARY TOXICITY

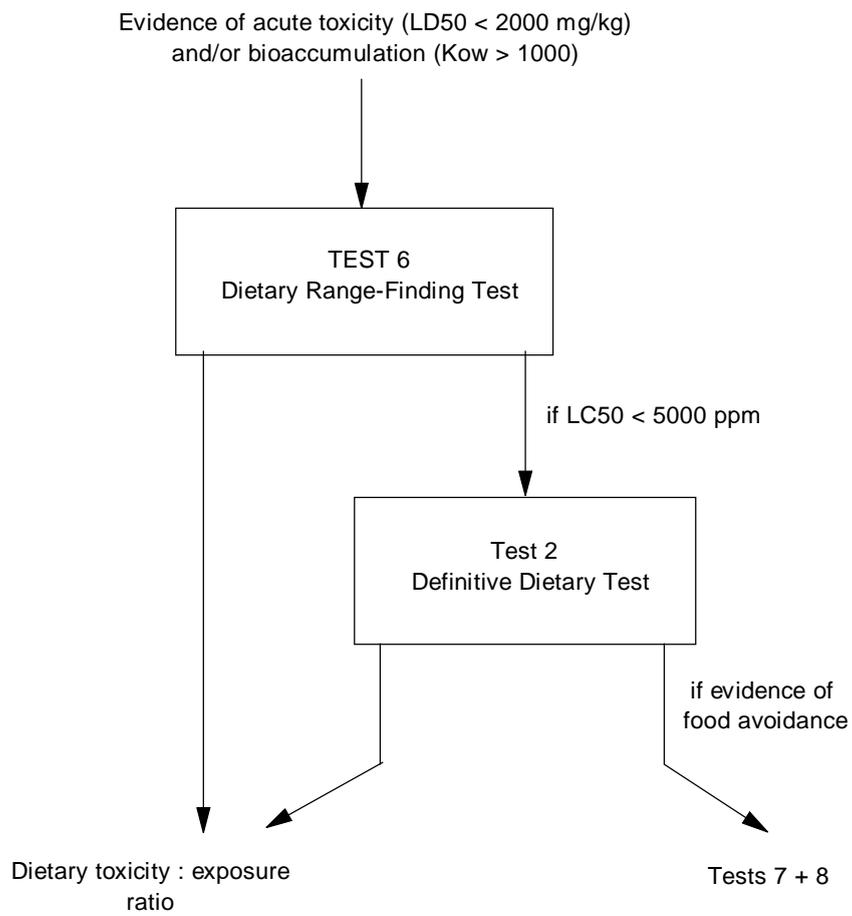


Figure 6.5

LATER TIERS - REPRODUCTIVE TOXICITY

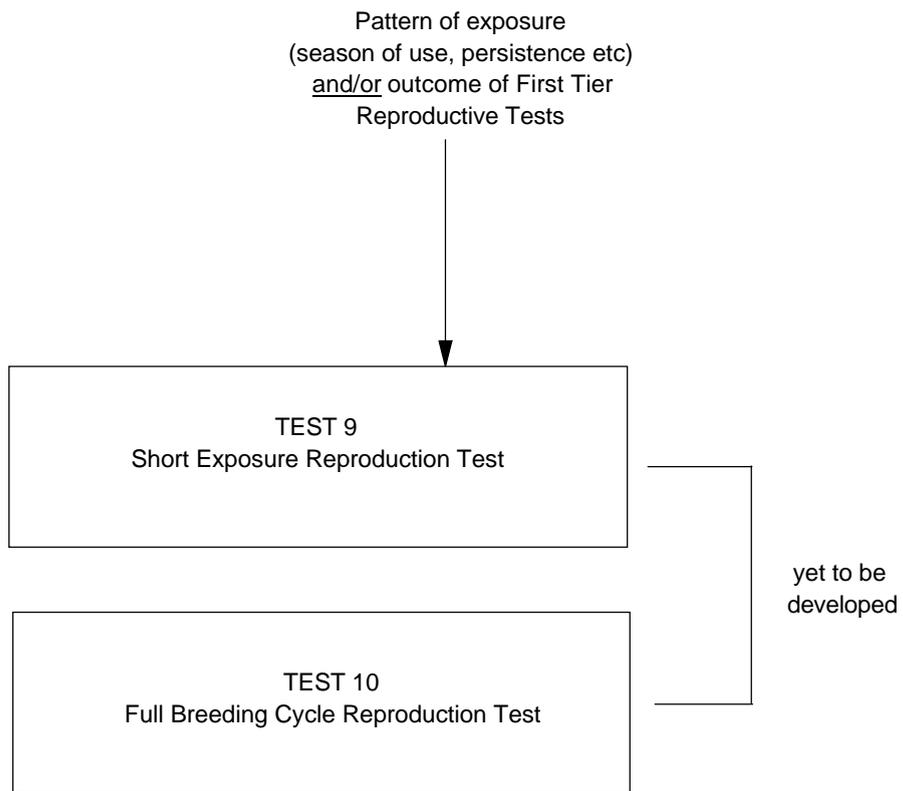
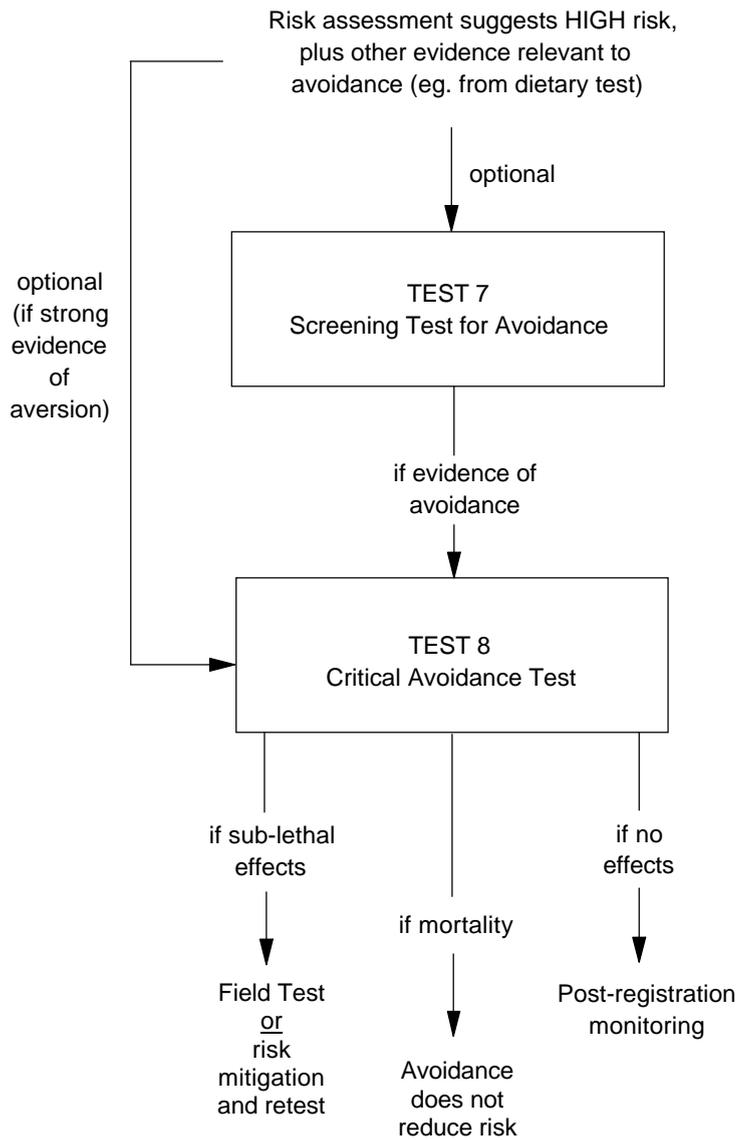


Figure 6.6

LATER TIERS - FOOD AVOIDANCE



7. Conclusions and Recommendations

This section draws together the major conclusions reached by the Workshop in addressing the objectives set out in the Introduction, i.e. 1) to define the need for toxicity data, the critical features of tests to generate these data; 2) to evaluate the positive and negative features of existing test methods, and of proposed alternatives, against the required critical features; and 3) to develop proposals for the revision and development of OECD Test Guidelines, and a framework for using them in risk assessment.

The Need for Avian Toxicity Data

The primary use of toxicity data was considered to be in the development of risk assessments based on toxicity and exposure data for the chemical. Risk assessments may be undertaken simply to determine whether a potential hazard may exist. However, they may be further refined by reducing uncertainty and producing a precise estimate of risk for a single species under a particular chemical use.

In addition, toxicity data may be used in the risk assessment process for purposes other than determining the final risk, e.g.:

- comparing toxicity data between species, chemicals and test conditions;
- setting triggers for further tests;
- range-finding for other studies;
- determining threshold levels at which effects occur;
- predicting food avoidance;
- predicting sub-lethal effects on health.

However, it was felt that, whilst addressing these objectives, the scale of testing should be minimised for reasons of economy and animal welfare. Therefore, tests should be designed within a framework which enables the scale of testing to be matched to the level of information required for the risk assessment.

Critical Features of the Tests

Features the Working Groups considered as necessary in all tests aimed at producing data suitable for use in risk assessment, included:

- Tests should be capable of predicting effects in the environment.
- The duration of a test should be appropriate to the persistence of the chemical in the field.
- Uncertainty in risk assessment, such as that due to extrapolation between species, should be minimised.
- Tests, whilst not compromising their scientific and statistical validity, should use the minimum number of animals.
- Tests should be applicable to pesticides and to general chemicals.

Problems of Current Test Design

A number of shortcomings in current test guidelines were identified:

Acute Toxicity Tests

- There is no current OECD Test Guideline.
- The limited number of species tested limits confidence in risk assessment due to interspecies differences in sensitivity.
- There is no guidance on routes of exposure other than oral.
- Regurgitation.

Dietary Toxicity Tests

- The duration of the existing test (5 days) does not accurately reflect patterns of exposure which occur in the field.
- Food avoidance is a confounding factor.
- The lack of replication limits the power to detect effects.
- The test design prevents good characterisation of sub-lethal effects.

Tests for Effects on Reproduction

- The current test design results in poor statistical power.
- The exposure profile does not correspond to the persistence profile for most current chemicals, particularly pesticides.
- The two species that are most often tested (bobwhite quail and mallard) are not the easiest to test.
- Lack of realism is rarely taken into account in risk assessment.
- There is no consideration of effects on the second generation.
- The current tests are large-scale, long and therefore expensive.

Tests for Avoidance

- There is no current OECD Test Guideline or guidance document.
- A number of differing methods have been published.

- The degree of avoidance is greatly affected by test conditions.
- There is uncertainty about the extent to which avoidance reduces risk in the field.

Proposed Tests, Tier Placement and Triggers

Three of the Working Groups recommended revisions and/or additions to current or proposed OECD Test Guidelines. The Avoidance Test Working Group decided that specific guidelines would not be appropriate for avoidance testing, at least at present, because the need for realistic conditions implies a need for wide flexibility in test design. Instead, the Group proposed the development of a guidance document identifying the options which exist for this type of study and the factors which should be considered in choosing amongst them.

Matching the amount of information actually required in the risk assessment to the level of testing is difficult if only a small number of tests are used to fulfil all aspects of data requirements. The Workshop approached this problem by devising a framework containing a basic set (first tier) of tests and a number of tests triggered by additional needs for data by the risk assessment, as described below.

Acute Toxicity Tests

1. A **limit test** on a single species at a single dose level (with an upper limit of 2000 mg/kg), to assess the need for a definitive LD50 test:
 - a first tier test;
 - trigger – any chemical for which there is potential exposure and which is not expected to be toxic.
2. A **definitive LD50 test** with adequate numbers and dose levels to characterise the median lethal dose (LD50), lowest lethal dose and slope:
 - triggered by mortality or adverse effects in the limit test;
 - triggered for a second species by limited knowledge of the properties of the chemical or high toxicity.
3. An **Up and Down test** may be used as a range-finding test for the definitive LD50 test, or to evaluate the approximate lethal dose for a wider number of species in order to reduce uncertainty in the risk assessment.
 - The trigger is feedback from the risk assessment if reduction of uncertainty is required.
4. It was felt that insufficient information was available to develop guidelines for **non-oral routes of exposure**. However, the Acute Toxicity Working Group identified a number of parameters (e.g. vapour pressure, partition coefficients) which might be used to trigger consideration of non-oral routes in risk assessment.

Dietary Toxicity Tests

1. A **range-finding test** to assess whether the LC50 is less than 5000 ppm and to indicate suitable doses for the definitive test
 - normally a second tier study triggered by LD50 and Kow data
2. A **definitive LC50 test** with adequate numbers and dose levels to characterise the the study's primary endpoints (mortality, body weight and food consumption)
 - triggered if the LC50 range-finding test shows that the LC50 is less than 5000 ppm
 - for one species, the most sensitive, if LD50 data are available for more than one species;
 - for a second species if the LD50 for only one species is available.

Tests for Effects on Reproduction

1. A **definitive test for effects on reproduction** with adequate numbers of birds and 3 dose levels to determine an NOEC, with suitable power, for the primary endpoint (production of viable chicks):
 - first tier study;
 - trigger — any chemical for which there is potential exposure of breeding birds.
2. A **second definitive test** for effects on reproduction using a different species, triggered:
 - if effects are seen in the test at below a limit level of 1000 ppm or 2 x PEC (whichever is the higher) and where testing in a second species is preferred to the application of a safety factor (to be determined, but likely to be between 10 and 100) to the NOEC;or
 - if no effects are seen at or below 1000 ppm or 2 x PEC (whichever is the higher) and where testing a second species is preferred to the application of a precautionary safety factor of 100 applied to the NOEC.
3. Other tests which may need to be considered, according to exposure profile and risk concerns:
 - **egg spraying or dipping test;**
 - **short exposure test;**
 - **full breeding cycle test;**
 - triggered according to the concerns relating to exposure to the chemical, and thus used to refine the risk assessment.

Tests for Food Avoidance

1. An optional **screening test** as a simple and sensitive measure of avoidance to decide whether conducting the critical avoidance test is warranted:
 - an optional test for pesticide uses for which a high risk has been identified,

- and for which more evidence is desired before proceeding with the critical test.
2. A **critical test** as a realistic and severe test which shows whether avoidance will mitigate risk under a fairly narrow range of conditions:
- triggered for pesticides where there is an assessment of high risk and there is reason to suppose this may be mitigated by avoidance, e.g.
 - a dietary test showing avoidance;
 - chemical structure similar to known repellents;
 - the optional screening test shows avoidance.

Species and Safety Factors

There are obviously a wide range of species which could be considered for use in toxicity testing. However, at present only three species are used routinely: mallard, and bobwhite and Japanese quail. Concerns have been raised about the extrapolation of data from these species to the wide range of species which may be exposed in the field. Several approaches to this problem were proposed by the Working Groups, including:

- testing of further phylogenically different and ecologically relevant species if there is a need to reduce uncertainty;
- testing of further species if other toxicity data are limited;
- option to use larger safety factors rather than test another species;
- use of field studies and post-registration monitoring.

It should be noted, however, that none of these options represents a general solution to the problem of extrapolation.

Animal Welfare

Animal welfare considerations were an underlying concern in all discussions on the need for tests and on their design. The major points resulting from these considerations were:

- Although there is a need to minimise both the number of animals used and their degree of suffering, the scientific integrity of the test should not be compromised.
- Testing should be avoided wherever extrapolation provides a reasonable alternative.
- The number of animals should be kept to the minimum necessary to ensure sufficient statistical power.
- Animal welfare should be considered when choosing species, group size, pen size, etc.

- There is a continuing need for information on lethality. The use of alternative endpoints may fail to prevent mortalities in the field, and may lead to further testing in the laboratory.
- With respect to acute toxicity, it was proposed that testing of low risk chemicals be minimised; however, for chemicals whose risk is more uncertain, it was recommended that more species be tested but using the Up and Down method rather than the definitive LD50 test.
- Birds should be removed from treatment if they show prolonged reductions in food intake to the point where they are at risk of starvation.
- Sub-chronic dietary tests are needed in addition to acute toxicity tests, due to differences in exposure scenarios.
- Information on other endpoints maximises information from testing and may reduce the need for further testing.
- Where possible, species (e.g. Japanese quail) which are less susceptible to self-inflicted injury in captivity should be used.

Recommendations for Test Revision or Development

Tables 7.1 to 7.3 outline the changes in current guidelines or development of new OECD Test Guidelines recommended by the Working Groups. As mentioned above, it is proposed to develop a guidance document, rather than a Guideline, for avoidance testing to allow flexibility and realism in test design. This is reflected in **Table 7.4**.

Recommendations for Further Work

A number of recommendations for further work were put forward by the individual Working Groups. These recommendations are summarised below.

Further Test Development and Evaluation

A number of the tests require further development and evaluation before they can be adopted as OECD Test Guidelines.

Acute toxicity tests

- The Avian Up and Down test procedure should be submitted to the OECD Test Guideline National Co-ordinators.
- A definitive LD50 study and a limit test should be developed.

Dietary toxicity tests

- The proposed dietary toxicity test design, using a 21-day exposure period of sub-adult animals, should be evaluated with particular consideration being given to whether fewer birds could be used at the lethal doses.
- If successfully developed, the new dietary toxicity test design should be ring-tested prior to adoption as a OECD Test Guideline.

Tests for effects on reproduction

- Inter-laboratory testing (ring-testing) of the proposed basic test guideline for avian reproduction in the Japanese quail should be performed in order to evaluate its performance, sensitivity, and reproducibility.
- The existing test protocols (especially for the mallard) should be reviewed in light of the proposed basic test design. Several new features of the basic Japanese quail test design are relevant to the mallard and other species.
- An additional test with short-term exposure to doses higher than currently tested should be developed for chemicals which are less persistent, and where parental toxicity may be expected to be high or dominant.
- Development of a full breeding cycle test in a passerine or other suitable species is required.

Tests for avoidance

- Many aspects of the methodology proposed for avoidance testing require refinement. In addition, further research is needed into the factors affecting avoidance in the field.
- Once these issues have been resolved, the refined methodology should be applied to a selection of existing pesticides and the results compared to (a) existing avoidance data from other tests, and (b) field studies and incident data.
- The need to draft more specific guidelines for particular types of avoidance test (e.g. for seed treatments) should be assessed.

Analysis of Existing Data

These recommendations relate to problems identified which may be addressed using existing data, e.g. in the selection of the most appropriate test species and numbers of test individuals to be used.

- There is a need to identify the appropriate number and identity of test species for acute oral toxicity testing and the utility of ALD (Approximate Lethal Dose) testing to provide adequate certainty in risk assessment, or, in the absence of testing of additional species, the appropriate safety factors. This should be undertaken by a group including representatives from Canada, Germany, The Netherlands and the United States (the originators of work to date).

- The most efficient way, in terms of cost and animal suffering, to generate a dose-response curve should be evaluated, with special consideration given to the numbers of animals used per treatment and the numbers of treatments.
- Field incident reports should be examined to assess the practical significance of non-oral routes of exposure. A database should be constructed on the effects of non-oral routes of exposure, to provide a basis for future guideline development.
- A comparative review of the sensitivity of juvenile and sub-adult birds to the toxic effect of chemicals in the dietary test should be conducted to ensure the recommended approach is correct.
- An analysis should be undertaken of all available data on the relative sensitivity of mallard, bobwhite and Japanese quail, to reproductive toxicants. If available data are insufficient to assess the relative sensitivity of these species, compounds identified as having caused reproductive effects in the mallard and bobwhite quail should be tested in the Japanese quail, possibly through incorporation in a ring-testing exercise.
- Existing data from dietary studies, avoidance studies, field studies and poisoning incidents should be reviewed to assess the reliability of laboratory-to-field extrapolation of avoidance, and to consider whether existing approaches are adequate for these purposes or whether further development is required.

Statistical Needs

- Statistical analysis is needed to refine the design of the avian reproduction test proposed here, so as to ensure maximum power while minimising the number of animals tested. In addition, the Working Group recommends that appropriate statistical methods be established and made standard for analysis of all test results.

Research Needs

Fundamental research needed in order to fill gaps in present knowledge was also identified.

- More work is needed on biomarkers for endocrine disruption, both in adults and the F1. Measurements of mixed function oxidase (MFO) enzymes may also be useful, given recently documented correlations between elevated levels of some enzyme families and endocrine effects.
- Although a discussion of how predicted environmental concentrations (PECs) should be determined falls outside the mandate given to the Workshop, determination of a PEC remains central to both the proposed tier progression and the interpretation of the results of the avian reproduction study.
- Field validation of reproductive effects seen in the laboratory.

- Circumstances under which repellency fails to protect against intoxication (e.g. when ingestion rate is high) should be investigated, and ways of incorporating these circumstances in test design should be developed.
- The contribution which sequential exposure makes to risk in the wild should be assessed. If this contribution is significant, consideration should be given to how it could be included in avoidance testing.

Table 7.1 Recommended Changes to the Acute Oral Toxicity Test

Parameter	Present (German draft proposal)	Proposed changes		
		Limit test	Definitive LD50 test	ALD test
Data provided	NOEL, LLD, LOEL	trigger for definitive test	LD50, LLD (LOEL optional)	ALD
Species tested	Japanese quail	mallard, bobwhite or Japanese quail	mallard, bobwhite or Japanese quail,	unrelated, preferably ecologically relevant species
Number of animals	6 per dose	3-5 each of males, females	lower but not defined	2 per dose
Dose levels	minimum 3	single	adequate to define LD50, LLD and slope	minimum to provide ALD
Light duration	>8 hours	8 hours	8 hours	8 hours
Age	over 6 weeks	full grown, in their first year and not in breeding condition	full grown, in their first year and not in breeding condition	single sex, young adults
Acclimation period	7 days	14 days	14 days	14 days for captive bred or 30 days for wild caught birds
Fasting period	15-20 hours	12-15 hours	12-15 hours	12-15 hours
Dosing vehicles for water insoluble substances	range	corn oil	corn oil	corn oil
Minimum observation period	14 days	14 days, but 21 days if mortalities are observed in the last 3 days	14 days, but 21 days if mortalities are observed in the last 3 days	14 days

Table 7.1 Recommended Changes to the Acute Oral Toxicity Test (continued)

Parameter	Present (draft proposal)	Proposed changes		
		Limit test	Definitive LD50 test	ALD test
Mortality monitoring	daily	continuously for the first 2 hours, at least twice in the remainder of the first day, and daily thereafter	continuously for the first 2 hours, at least twice in the remainder of the first day, and daily thereafter	continuously for the first 2 hours, at least twice in the remainder of the first day and daily thereafter
Behaviour monitoring	daily	continuously for the first 2 hours and daily thereafter	continuously for the first 2 hours and daily thereafter	continuously for the first 2 hours and daily thereafter
Additional observations		regurgitation monitored continuously for the first 2 hours	regurgitation monitored continuously for the first 2 hours	regurgitation monitored continuously for the first 2 hours
Food consumption	average daily consumption		monitored on days 1, 3, 7 and 14	
Gross pathology	all animals		all animals dying and 4 randomly selected survivors from each treatment group and controls	

Table 7.2 Recommended Changes to the Dietary Toxicity Test

Parameter	Present (OECD 205)	Proposed changes
Data provided	LC50, NOEC, LC100, slope of concentration-response curve	LC50, food consumption data
Species	mallard, mobwhite quail, pigeon, Japanese quail, ring-necked pheasant, red-legged partridge	Bobwhite quail or mallard preferred, ring-necked pheasant or red-legged partridge possible, not Japanese quail
Test period	5 days	21 days
Recovery period	3-18 days	at least 7 days - longer if birds are still dying during this period
Housing	group housed	individually
Age	10-17 days	42± 2 days
Light duration	12-16 hours	8 hours
Caloric content of diet	not determined	determined
Fasting	not stated	overnight
Highest dose	not stated	results in approx. 85% mortality
Number of birds per test dose	10	16 at the highest and lowest doses, 10 at other doses
Control group	10	14
Food consumption	days 0, 5, 8 and end of test	daily
Body weight	days 0, 5, 8 and end of test	weekly from acclimation to the end of the recovery period
Clinical signs	twice on day 1 and then daily	twice daily
Clinical chemistry	not monitored	5 birds per dose, (a) between days -5 and 0, and (b) on day 21
Gross pathology	not monitored	all birds that die, and gross pathology and organ weights on half the surviving birds at day 21
Histopathology	not monitored	all birds that die, on 50% control birds and dosed group showing 50% mortality, and at end of recovery period for birds exhibiting adverse effects during-test

Table 7.3 Recommended Changes to Tests for Effects on Reproduction

Parameter	Present (OECD 206)	Proposed changes
Data provided	NOEC	NOEC with stated power
Species	bobwhite quail, Japanese quail, mallard,	provisionally Japanese quail first test, mallard optional second species
Study length	at least 20 weeks	6 weeks
Pre-lay exposure period	yes	no
Dose levels	highest approx. 50% LC10	highest dose set to observe effects with upper limit of 1000 ppm or 2 x PEC, whichever is the higher
Age	Japanese quail: proven breeders; bobwhite quail: 20-24 weeks; mallard: 9-12 months	proven breeders 2-3 weeks after initiation of laying (just starting peak egg production)
Controls	control group	and 2 week pre-dose data as a covariate
Removal of infertile or poorly reproducing pairs	not done	done to increase overall statistical power
Exposure period	20 weeks	at least 6 weeks
Description of diet	source, composition, nutrients	and caloric content, including carrier
Egg collection period	last 10 weeks	all eggs
Egg candling	to detect cracks	to differentiate between infertility and early embryo death
Reproductive parameters	eggshell thickness, cracked eggs, egg production, eggs set, viability, hatchability	and eggshell breaking strength
Chick parameters	survival of young, body weight (14 days), food consumption (weeks 1 & 2)	and gross examination of gonads and sex determination of chicks at highest dose (if effects found, examination of other dose groups and histopathological examination when effects found); option of assessing reproductive viability of F1 chicks at breeding age
Adult parameters	mortality, toxic signs, body weight (beginning, prior to onset of laying, and end), food consumption, gross pathological examination for all birds	and necropsy and gross pathology of all animals, wet weight of liver, spleen, male gonad, histopathology on organs showing changes, body weight at initiation of dosing and end

Table 7.4 Recommended Outline for Tests for Food Avoidance

Parameter	Screening test	Critical test
Data provided	relative consumption of treated and untreated food under standard conditions	mortality and sub-lethal effects under realistic conditions but tending to worst case
Species	surrogate species, e.g. bobwhite, Japanese quail or mallard, or representative species, e.g. red-winged blackbird, starling, rock dove	species most at risk is recommended, but if impracticable or unacceptable a representative surrogate species, e.g. ecologically and metabolically relevant
Controls	not required	as treated but undosed
Accommodation	individual, visually isolated	ecologically relevant
Age/sex	adults, both sexes	ecologically and metabolically relevant
Acclimation	5-7 days	5-7 days
Pre-treatment period	4 days	pre-exposure to untreated substrate in acclimation period if ecologically appropriate
Test material	technical active ingredient mixed in standard diet	formulation on ecologically relevant substrate
Treatment levels	5, lowest below expected LOEL, highest above discrimination threshold	probably up to 4, ecologically relevant including maximum in field
Fasting	simulate normal diurnal restrictions	simulate normal diurnal restrictions
Treatment period	4 days	to ensure a lethal exposure if ingested, usually 4 days
Post treatment period	4 days	4 days
Presentation of food	1 day's requirement in food cups or trays	representative of field exposure: spreadout on the substrate
Choices	treated and untreated food	representative of field exposure; continuous simultaneous choices of treated and untreated material
Measurements	feed consumption, body weight, mortality, sub-lethal effects, environmental conditions, residue in feed	mortality, sub-lethal effects, body weight, feed consumption (if practical), environmental conditions, residue in feed

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Annex 1

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Annex 2

Questionnaire: Request for Information and Opinions from Participants

In order to help organise the programme for the Workshop, and to obtain maximum benefit from participants' expertise, participants were requested to complete the questionnaire below prior to the Workshop.

The questionnaire sought views on key aspects of acute oral toxicity, other routes of acute exposure, dietary exposure, avoidance (palatability and acceptance) and reproductive effects, as well as some more general issues that were to be discussed in plenary sessions. The aim was to assemble as complete a picture as possible of what was going on in the field of avian toxicity, and what issues experts considered to be critical at the moment.

Responses to the general questions are reported in Annex 3.

.....

Questionnaire

Background information

1. Name:
2. Organisation:
3. Are you involved in regulatory activities? Please give a brief outline.
4. Have you carried out avian tests? Please give a brief outline.
5. Are you involved in research on avian toxicology? Please give a brief outline.

General issues

6. Do you feel that there is a continuing need for guidelines on the following aspects of avian toxicity, to aid the regulatory assessment of pesticides:
 - acute oral toxicity?
 - other routes of acute exposure?
 - chronic dietary toxicity?
 - palatability and acceptance?
 - reproductive effects?
7. Should any additional aspects be the subject of a guideline, either for pesticides or for other substances that might require avian tests?

8. Should testing be carried out using active ingredients or formulations (in the case of pesticides)? For general chemicals, should tests be on individual substances or on mixtures/preparations?
9. Should tests include the mediating effects of age and sex differences, environmental factors (e.g. temperature, nutritional stress) and other sources of variability within species?
10. What steps could be taken to improve the welfare of subjects in avian toxicity testing? In particular, should the design of tests be modified to prevent suffering, starvation and injury, at the expense of the information provided by the test?

Acute oral toxicity

11. Are you content with currently available guidelines for acute oral testing? (These include EPA Guideline 71-1; OECD draft guideline; FAO draft revised guideline 2.1.1.)
12. If not, what are the shortcomings, and what would be an 'ideal' design? Please comment on each of the following aspects, and any others you feel are relevant.
 - test species and the age of subjects
 - endpoints for measuring effects
 - conditions in which tests are carried out
 - preparation and administration of doses
 - numbers of animals tested, number of treatment levels, replication
 - duration of observation periods
 - attention to animal welfare
 - adherence to GLP
 - others?
13. Could this type of test be combined with other aspects of avian toxicity testing? Is it desirable to do so?
14. What role do you consider that acute oral testing has in risk assessment? Should it be confined to initial screening purposes, or extended to other applications (e.g. to explore species differences in sensitivity)?
15. Could toxic effects be measured in more appropriate or economical ways than the current endpoints (LD50, NOEL)? For example, do you favour the use of fixed dose procedures, benchmark testing, or criteria based on partial effects (EC10, EC15, etc) rather than no-effect levels?
16. Do acute oral tests allow extrapolation of toxicity between species? Are there developments you would wish to see to enhance this, such as standard databases, mechanistic models, or empirical relationships for extrapolation?

Other routes of acute exposure

17. What guidance exists for the assessment of the effects of exposure through routes other than dietary intake or acute oral dosing (e.g. inhalation, dermal absorption)? Is it adequate? If not, what aspects require development?
18. Is there a need for these aspects to be the subject of standard guidelines, or should they be addressed by more flexible, ad-hoc studies?
19. Should they be integrated with results of tests on acute oral toxicity, and if so, how?
20. Could this type of test be combined with other areas of avian toxicity?

Dietary toxicity

21. Are you content with currently available guidelines for dietary toxicity testing? (These include OECD Guideline 205)
22. If not, what are the shortcomings, and what would be an 'ideal' design? Please comment on each of the following aspects, and any others you feel are relevant:
 - number of treatment levels
 - number of animals tested at each level
 - duration of the exposure period
 - replication
 - endpoints used to measure effects
 - procedure for presenting food to birds
 - others?
23. To what extent should this type of test be designed to reproduce ecologically realistic conditions (e.g. species, mode of exposure)?
24. How many species should be tested, and which particular species or types of birds are appropriate?
25. Do dietary toxicity tests allow extrapolation between species? Are there developments you would wish to see to enhance this, such as standard databases, mechanistic models, or empirical relationships?
26. How can laboratory tests of this type be related to birds living under field conditions? Please comment on each of the following, and any other aspects:
 - caloric content of food
 - metabolic rate
 - food assimilation efficiency
 - assimilation of toxic compounds
 - other factors affecting sensitivity
 - others?
27. Could this type of test be combined with testing for other aspects of avian toxicity?

Palatability and acceptance of food

28. In what circumstances are data on food avoidance (whether due to repellency, conditioned aversion or anorexia) required, and what regulatory questions should they address?
29. Are you content with currently available approaches to assessment of food avoidance? Are standard guidelines required, or can the information be obtained from food intake/body weight data from standard dietary toxicity tests? What are the advantages and disadvantages of those alternatives?
30. If you consider that a guideline is desirable, what would be an 'ideal' design? Please comment on each of the following aspects, and any others you feel are relevant:
 - number and setting of treatment levels
 - number of animals tested, and division into groups
 - housing and other conditions
 - duration of the test
 - observations to be made as well as the primary endpoints
 - endpoints to measure effects, and their statistical treatment
 - others?
31. Should choice tests or no-choice tests be used, or both?
32. What evidence exists to indicate whether any existing measure of food avoidance by captive birds provides a reliable prediction of avoidance in the wild? What developments would you wish to see to enhance the ability to extrapolate between species, and to conditions in the field?
33. Could this type of test be combined with any other aspects of avian toxicity testing?

Reproductive effects

34. Are you content with currently available guidelines for testing reproductive effects on birds?
(These include OECD Guideline 206, EPA guideline)
35. If not, what are the shortcomings, and what would be an 'ideal' design? Please comment on each of the following aspects, and any others you feel are relevant:
 - lack of realism (e.g. artificial incubation)
 - the duration of the test
 - statistical power (design, replication, etc.)
 - choice of species and number of species tested
 - endpoints for measurement of effects, particularly the use of
 - biochemical and histopathological
 - restriction of testing to the F1 generation
 - ease of interpretation and analysis of results
 - setting of dose levels, and number of levels
 - others?

36. In your opinion, what is the primary reason for conducting an avian reproductive test? Please rank the following reasons:
- to simulate avian reproduction under realistic conditions of exposure
 - to show a substance's potential to affect the reproductive system of birds
 - to act as a possible trigger for field studies and help in their design
 - to simulate a chronic exposure situation, and define a maximum tolerated concentration for birds
37. Assuming you believe in the usefulness of an avian reproduction study, how should it be triggered? Please rank the following statements:
- The test should always be done.
 - It depends on data on mammalian reproduction and/or metabolism.
 - It depends on likely exposure of birds to the substance (e.g. acute vs chronic).
 - It depends on the results of avian LC50 and LD50 tests.
 - It depends on the type of chemical (e.g. pesticides vs other substances).
38. Could this type of test be combined with other aspects of avian toxicity testing?
39. Could effects on avian reproduction be adequately assessed without carrying out tests, for example by relying on mammalian test results or other non-avian data?

Annex 3

Summary of Responses to the Pre-Workshop Questionnaire: General Issues

This Annex summarises the responses to the general questions in the "Request for information and opinions from Participants" sent to all prospective participants in the OECD/SETAC Workshop on Avian Toxicity Testing (see Annex 2). Responses to other questions in each section were used by the relevant group leaders in preparing for the Workshop. A list of respondents is given in Appendix 1 to this Annex.

The following is a summary of responses to questions 6-10, and the general questions in the sections on acute oral tests (11), dietary tests (21), food avoidance tests (29) and reproductive tests (34). Comments are given verbatim. The order in which these comments are presented is random and expresses no order of preference. Numbers in brackets indicate the number of times substantially the same comment was made in more than 1 response.

6 Do you feel that there is a continuing need for guidelines on the following aspects of avian toxicity, to aid the regulatory assessment of pesticides:

- acute oral toxicity?
 - other routes of acute exposure?
 - chronic dietary toxicity?
 - palatability and acceptance?
 - reproductive effects?
-

Need for guidelines	Yes	No	Maybe
Acute oral toxicity	30	1	1
Other routes of acute exposure	19	9	3
Chronic dietary toxicity	27	4	1
Palatability and acceptance	32	0	0
Reproductive effects	32	0	0

General comments

1. Need for guidelines covering both the conduct of these studies and their interpretation and use in risk assessment.(3)
2. Continuing need for developing and improving guidelines for toxicity tests in all of these categories, with emphasis on how the test results relate to field.
3. Continuing need for guidelines in all the areas with greater emphasis on the collection of data on sub-lethal effects, behavioural and biochemical/physiological. Such data should allow comparability between compounds and extrapolation between species as well as improve understanding of pesticide mechanisms of effect and prediction and interpretation of effects under field conditions.
4. Some of the laboratory tests could probably be replaced by semi-field tests, where the different aspects could be tested simultaneously under the same exposure conditions.

5. Guidelines reduce the difficulties inherent in product registration. Also, they permit a database that can be used to compare the toxicity (etc.) of different pesticides.
6. We need to define the questions to be answered.
7. Are we trying to:
 - improve the accuracy and precision of avian toxicity tests?
 - rank the relative toxicity of test substances to avian species?
 - reduce costs of tests without sacrificing accuracy or precision?
 - reduce numbers of animals without sacrificing accuracy or precision?
 - develop better methodologies for new risk assessment models?

A tremendous amount of the avian work that has been performed is in response to regulatory requirements and questions, and like most investigators I feel that there are some changes in details in the standard EPA and OECD guideline avian tests that should be addressed. However, within the context of current regulatory risk assessments, I am not dissatisfied with the overall approach to avian toxicity testing. My concern is that we have "the cart before the horse" and that this group of experts should really be addressing our overall testing goals before we re-evaluate the adequacy of current test procedures.

In the past, a significant amount of avian toxicity testing focused on the performance of a few standardized tests that were used in relatively crude risk assessments. While such assessments have served us well for the vast majority of test issues to date, it has become increasingly apparent that newer and more refined risk assessment procedures are becoming necessary to meet today's scientific and regulatory needs. It would seem prudent to evaluate current standard tests in view of our current and near-future capabilities in risk assessment.

Specific comments on each tests were:

Acute oral toxicity

1. Acute oral toxicity is an essential measure to cover regulation of applied pesticides with short term effects.
2. EPA guideline needs only minor (if any) modification on a case-by-case basis, and when treated as a guideline, rather than a protocol, the flexibility to do so already exists within the guideline.
3. See German proposal for an OECD-guideline 205 'Avian Acute Toxicity Test - Oral Toxicity in the Japanese Quail'.
4. More species, fewer animals, ALD.

Other routes of acute exposure

1. Difficult to see the need for tests on other routes of exposure of birds.
2. Mammalian studies can be used to identify if other routes of exposure (dermal and inhalation) need to be investigated further in birds. Research may be necessary to check if mammals are satisfactory indicators for inhalation and dermal toxicity in birds.
3. Possibly dermal and inhalation exposure in specific cases, since oral exposure is only one route. (2)
4. Yes, but on a case-by-case approach, not as standard tests.(2)
5. Dermal (skin or feet), appropriate species only, limited animals, ALD.
6. Currently tests by non-oral routes are not requested frequently enough by regulatory authorities to warrant standard methods, though I feel the need for more attention to these routes should be reviewed.

Chronic dietary toxicity

1. Unlikely to be of use for modern pesticides which are increasingly less persistent in the environment, at least on food of birds.
2. The sub-acute dietary study guideline does not adequately fulfill this role and should be discontinued or at least retained as an optional study. A new chronic test must have a duration of exposure relevant to at least the duration of exposure expected on diet in the field.
3. Badly interpretable results where food consumption and body weight gain are concerned, caloric value of diet not comparable to that in the field.
4. OECD 205 can be developed to provide more useful information without much increase of work involved.(2)
5. Only in combination with other aspects (e.g. reproduction), if it means an approx 6 week feeding period.
6. 5-day and 30-day, quail and other species as appropriate, limited animals, ALC.
7. The major benefits of sub-chronic dietary tests and palatability/acceptance tests are that they may provide some insight into exposure and toxicity as modified by behavioural responses to contaminated food. Research is required to assess whether these tests are reliable predictors of responses in the wild, or whether they can be improved upon. If reliable tests cannot be developed, then no guidelines should be maintained. Otherwise, guidance of some sort will be desirable, especially as test design seems likely to be critical to predictive value. However, it may be that the guidelines will have to be highly flexible to ensure relevance in each case.

Palatability and acceptance

1. There are cases where laboratory studies and field observations do not match in this respect.
2. Useful studies for research into improving formulations to lower the risk of exposure, especially for baits and seed treatments. Ad hoc studies are probably sufficient.
3. Only as a next step after acute oral toxicity. The current BBA guidelines should be improved on.
4. Perhaps this item can be included in the OECD 205 guideline.
5. Taste tests needed e.g. concerning grain like products and seed dressing chemicals.
6. Development of guidelines would aid regulatory assessment of seed dressing pesticides and granular formulations.
7. Considering the importance of this for ag-chem products, development of test guidelines is more than desirable. (2)
8. See German BBA-approach 25-1 'Testing of baits, granules and treated seed for hazards to birds - acceptance tests'
9. Choice and no-choice, appropriate species only, limited numbers, AED.

Reproductive effects

1. Crucial for any understanding of the ecological significance of effects of pesticides on birds. It is the reduction in reproductive efficiency which is most likely to have effects at the population level. Isolated acute toxicity incidents usually have little or no ecological impact.
2. Should be seen as a chronic dietary toxicity guideline with a duration of exposure relevant to at least the duration of exposure expected on diet in the field.
3. Similar or more useful information can be obtained with reduced resources in terms of animal sacrifice, costs and time.
4. See German proposal for OECD-guideline 206 'Avian Sub-chronic Toxicity Test - Oral Toxicity Including Effects on Reproduction in the Japanese Quail'
5. 30-day only, quail and other species as appropriate, male and female, limited numbers, AED.

7 Should any additional aspects be the subject of a guideline, either for pesticides or for other substances that might require avian tests?

	Yes	No
Additional aspects required	13	13

Guidelines specified	
Risk assessment procedure (2)	Guidance for the interpretation of test results from one test in light of results from other tests should be provided (e.g. LC50 combined with LD50). Guidelines on all aspects of the risk assessment process.
Mitigation and monitoring (1)	Guidelines on all aspects of the risk assessment process including: basic tests, risk assessments, mitigation, monitoring etc.
Tiered testing (1)	Perhaps a guideline which gives information on which tests are necessary for a certain tier and which information triggers additional testing. This will minimize suffering of test animals.
Collection of sub-lethal data (2)	Standardised methods for the collection of sub-lethal data, e.g. behavioural measurements and biochemical assays to ensure inter-laboratory comparison. Sub-organ effects when necessary.
Behaviour (1)	Behaviour effects.
Immune competence (1)	Immunotoxicity.
Estrogenic effects (1)	An additional guideline may be needed for effects of estrogenic activity of pesticides.
Dermal and inhalation (1)	For highly toxic materials, oral LD50 <25 mg/kg, dermal testing should be considered and a guideline is needed. I believe that inhalation studies are needed less often; this should be a discussion point.
Sub-chronic (1)	An avian equivalent of the rodent 90-day sub-chronic test, which is an excellent surrogate for chronic effects (except for oncogenicity, which in this context would not normally be of particular concern). The sub-chronic test is usually conducted over what would be about 10% of the organism's life-span. Sub-chronic and reproductive effects (and behavioral and neurotoxicity testing) can also be combined.
Second generation effects	The second generation effects of pesticides on birds should be examined and, as such, guidelines should be established for this purpose.
Repellant additives (1)	Repellent additives to dangerous pesticide formulations can reduce non-target hazards to birds. There should be guidelines regarding the evaluation of these additives.
Secondary poisoning (3)	Studies where poisoned rodents are fed to predators are necessary in the case of some rodenticides; a guideline is currently in progress by EPPO. It is always difficult to estimate effects of rodenticide as for secondary poisoning, if all that is available is diverse field studies, monitoring data, etc. Suggestions for standard field tests would be useful. Guidance (as opposed to guidelines) for secondary poisoning studies.
Field tests (2)	Regarding field tests there should be some general guidance, but no strict guideline. Guidance (as opposed to guidelines) for field studies; would require a major effort to reach consensus.

General comments

1. Other aspects of the subject are important, but probably not developed enough to be captured in a 'guideline'.
2. Yes, for special cases, or special (new) chemistries. No for typical cases or typical chemistries (e.g. grow out F1 of avian reproduction studies to measure morphology of mature organs).
3. Further testing should be seen as optional and ad hoc methods used to evaluate specific questions.
4. The guidelines should focus to major risks, not to exceptional cases. It is not economic to develop guidelines just to address minor risks or exceptional situations.

8 Should testing be carried out using active ingredients or formulations (in the case of pesticides)? For general chemicals, should tests be on individual substances or on mixtures/preparations?

Pesticides	Number
Only AI,	3
Only formulation	6
Always Both	3
AI, formulation compound dependent ¹	10
AI, formulation test dependent ²	8
General chemicals	
Individual	2
Mixture	7
Both	2
Compound dependent	4

¹ Decisions on testing the formulation should be dependent on the characteristics of the chemicals in the formulation.

² Decisions on testing the formulation should be dependent on the type of test undertaken.

Pesticides

1. For chemicals with high toxicity, consideration should be given to testing representative formulations.(3)
2. For long-term reproduction tests, I would think that the tests should continue to be conducted with the active ingredient. For the short-term tests, having information on both would be best, but if only one were feasible, it seems that for granular products the granule should be used and that for determining palatability and acceptance it may be best to evaluate the formulation.
3. There is a need to develop a toxicity database for additives to formulated products for general reference.
4. Oral exposure occurs mainly to a.i., thus oral toxicity testing should be done with the a.i. If dermal exposure is relevant in contributing to overall toxicity, testing with the formulation (spray liquid) may be preferable.
5. Lower tiered studies on active/single substances, higher tiered studies on end-use products.(8)
6. We need to agree one or the other to avoid repeating studies (EC 91/414 Annex III).

7. Formulations should only be required, if there is good reason to believe that in use the results will be different from extrapolated values for the formulation (i.e. changes in palatability or penetration). Field test monitoring can confirm or refute laboratory data.
8. Familiarity with a particular chemical class or the "inert" ingredients of a formulation may allow only one of these to be tested. However, in the case of pesticides, where the formulation is designed to enhance a chemical's effectiveness in some way, both the active ingredient, by itself, and the formulation may need to be tested, at least in the lower testing tiers.
9. Testing should be carried out using formulations in order to assess the synergistic effects of pesticide combinations both with carriers, e.g. causing increased uptake rate, and other co-formulated pesticides, e.g. fungicides & herbicides.
10. Synergistic effects can be seen in mammal toxicity studies, which are performed for formulations. Thus, formulation testing should be restricted to these cases, where a significantly higher toxicity for the formulation in mammalian toxicity testing was found and this would change the outcome of a risk assessment for bird.
11. A pesticide formulation may dramatically alter test results, and should be tested; or it may be useful to understand the toxicity of one or more constituents in a mixture. It is always best to evaluate the physical-chemical nature of a test substance in relation to the potential for avian exposure before determining whether an active ingredient, formulation, or chemical mixture is to be tested.
12. There is merit in testing formulations - at least the non-volatile elements which may remain in the environment and modify the behaviour of the a.i. The acute toxicity of formulations has been shown to differ substantially from that of the technical. However, the acute toxicity of formulations is already tested in lab rodents - perhaps what is needed is a correction factor, based on the mammalian data, but applied to the avian results.
13. Preliminary screening should be done with the active ingredient. Where this reveals a potential hazard, consideration should be given to conducting additional tests with the formulation if its properties are thought likely to influence risk significantly. This may most often apply to palatability tests in cases where birds may feed directly on the formulation, but palatability tests may also be conducted with the active ingredient to assess responses to natural foods contaminated by spraying or other means. I would like to see testing of mixtures but targetted on combinations which are likely to cause significant synergism. Otherwise, additive effects should be assumed.

General chemicals

1. Testing mixtures (general chemicals) is impracticable in most cases because of resource limitations.
2. Individual substances for general chemicals unless there is indication from mammalian studies for synergistic effects
3. The technical product should be used in most cases. In instances where potential exposure to birds may occur and where it may exceed levels of concern, mixture/preparation testing may be useful.
4. I would like to see testing of mixtures but targetted on combinations which are likely to cause significant synergism. Otherwise, additive effects should be assumed.

9 Should tests include the mediating effects of age and sex differences, environmental factors (e.g. temperature, nutritional stress) and other sources of variability within species?

	Yes	No	Maybe
Include mediating effects	10	15	6

Include mediating effects

1. Age (4) and sex (5) differences definitely; the other mediating effects possibly. (2)
2. I do not think it would be practical to routinely try to account for other sources of variability in avian toxicity tests.
3. At least the age and sex differences. Note, however, that these cannot be separated from other sources of variability, like bioenergetics, when growing and/or reproducing animals are concerned.
4. Testing should be done in such a way as to include as much sensitivity to normal physiological factors as possible (e.g., both male and female birds should be tested; young birds are often more sensitive, etc.). The incorporation of environmental stresses (starvation, heat, cold, etc.) should not normally be done unless there is good reason to expect this type of scenario to typically occur. It would also add too much complexity/ confounding to the tests as they are now designed or would mean an (unnecessarily burdensome) addition to the present test protocols. (2)

Do not include mediating effects

1. The possible permutations and combinations would make this impractical. (5)
2. It is more appropriate to tackle these in the field. However, we might pay more attention to some unrealistic aspects of current testing - e.g. the high caloric value of lab chow - to see how this may affect the end results. The age of the test animals is also a concern.
3. Tests must be designed to be reproducible. Introducing environmental stresses will increase the variability of the results and could then reduce the power of the study.
4. The requirement for 'wild-type' avian species in toxicity testing results in considerable biological variation even under standardized experimental/environmental conditions. For this reason the generation of reliable and reproducible avian toxicity data for risk assessment must be considered as a relevant problem. Therefore, variation or additional inclusion of the above parameters in avian toxicity testing would probably not yield valid data necessary for regulator decisions.
5. Build-in safety factors of several risk assessment procedures to cover possible higher sensitivity of young or stressed animals. (3) However, this implies a need for rigorous validation.
6. Separate tests may be initiated to evaluate any number of factors that may mediate the toxicity of a chemical substance.
7. They should be determined in a general way (some bridging studies already exist) and then considered in the process of hazard/risk assessment.
8. Particular care is needed in specifying the standard conditions in cases where they may have a substantial effect on the results. This may be the case for dietary and palatability tests, where consumption may be significantly increased under levels of nutritional stress which are rarely seen in laboratory animals but may be normal in the wild.

Maybe

1. Only if the question at hand directly dealt with the variable responses caused by differences in these factors.
2. A bird which is entering reproduction is fit to do so. Individuals low in the hierarchy, suffering food stress, etc. will either die in the wild or will fail to reproduce. Reproductive failure of unfit individuals occurs at an early stage and is quite different from poor performance later in the cycle. My problem with present reproduction testing is that it does not represent the "worst case" scenario for breeding birds. In the wild, the exact time of nest building or egg laying is variable depending on local environmental conditions of temperature, rainfall, etc. It is common to see variations of days or weeks between years in local populations. They are, therefore, poised to reproduce in conditions which are, by definition, marginal for success. The photoperiod at this time is usually just long enough to initiate breeding in the wild. Tests do not reflect the marginality of breeding conditions, which can easily be overridden at long photoperiods. We have reviewed and researched the role of food consumption at this critical time.
3. Research into the impact of mediating parameters on risk assessment and uncertainty must precede revision of guidelines.
4. Not in lower tiered studies, but the possibility should be there for higher tiered studies. (2)
5. Testing to evaluate any of these parameters should be specific to the possible variability factor. A broad, shotgun approach is not practical and necessary and it will not usually yield any useful results.

10 What steps could be taken to improve the welfare of subjects in avian toxicity testing? In particular, should the design of tests be modified to prevent suffering, starvation and injury, at the expense of the information provided by the test?

No need to modify tests	1
Design of tests should be modified	29
Case-by-case decision	1

General comments

1. The check list approach does not reflect current thinking.
2. Isn't it self-evident that test birds should not suffer unnecessarily? This is a basic principle in herbicide-PPP directive.
3. Animal welfare aspects are important, and suffering should be kept to a minimum. (2)
4. It would be necessary to apply a scientific effort to gain a better comprehension of the welfare of subjects.
5. Testing must reflect the standards of animal welfare and human-induced suffering upheld in the real world. For example, if it is suspected that animals undergo starvation and temporary debilitation in the field following exposure to the operational use of a pesticide, experimenters must be allowed to simulate these conditions in the laboratory and at least be able to ascertain whether survival is likely. We suspect that these effects are probably more common than pesticide-induced mortality. To ignore them would be scientifically irresponsible.
6. The design of experiments should minimize suffering and pain whenever possible. However, modifications should not be at the expense of information provided by the test if the information is critical. (3)

7. Welfare of test animals can greatly be reduced by not requiring data representing minor risk areas and not setting too strict triggers for higher tier studies (e.g. EU: a NOEL \leq 1000 mg/kg bw in acute oral toxicity triggers further studies with a considerable number of animals!).
8. It is also important that we try to minimize the use of test animals not only within study designs but by minimizing repeated testing. (2)
9. The welfare of the birds should be of paramount importance. At all times we should seek to reduce group sizes, number of groups, etc.
10. Balancing the alternatives given must probably be decided case-by-case.
11. There are recently some possibilities to modify or refine the test design which is not compared with an expense of information provided by the test. Such possibilities are included in the German proposals.
12. In cases where a chemical's toxicity is due, for instance, to caustic effects at (unrealistically) high doses rather than inherent toxicity, acute testing may be skipped in favor of slightly longer-term testing to avoid what would be both unnecessary testing and suffering.
13. Welfare could be improved by greater emphasis on the collection of meaningful sub-lethal data in order to assess closeness of exposure to a lethal dose without the need for prolonged animal suffering, e.g. use of ED50 appropriate to the chemical under study, such as brain AChE for OPs and carbamates. (2)
14. Research should be done to assess the feasibility (and reliability) of limiting the number of avian tests by screening out low-risk compounds using data for invertebrates, mammals (used for human risk assessment), QSARs, or a combination of these, or *in vitro* methods.

Possible improvements

Caging

1. In reproduction studies, Japanese and bobwhite quail should only be placed together (male and female) during a short period in the day to prevent injuries.
2. Ducks should be kept so that males cannot interact.
3. Better housing conditions would be of benefit to birds and test results.
4. Guidelines for housing and husbandry of birds should be written with consideration of the birds' welfare. No more than 2 birds should be housed in a cage to minimise injuries due to aggression. (2)
5. Cage design could be evaluated. A publication would be useful to disseminate information about bird maintenance tips such as: cage sizes, wire coatings (to alleviate foot ailments), rubber ceiling covers (to alleviate head injuries), food and water delivery systems. (2)
6. In general, the conditions under which birds are kept and their physical condition during tests, as witnessed in commercial and government laboratories, have been acceptably humane. The exceptions to this have resulted from the species tested, most notably bobwhite, that are combative and excitable, rather than poor housing conditions.

Numbers

1. Reduce number of individuals in acute studies and find threshold level for symptoms and mortality
2. There is probably scope for reducing the numbers of test animals through 1) recognising there is no further need for a particular guideline and 2) reducing numbers of replicates at the expense of precision. Considering the low precision in risk assessment, less precision in toxicity endpoint thresholds may be acceptable.

3. Use of a smaller directed study to determine the potential for effects on the young prior to a full reproduction test for those classes of chemicals that have repeatedly shown a lack of effects on the young.
4. Consideration should be given to up-and-down and (less likely to be appropriate for pesticides) fixed dose procedures, to reduce numbers of animals used. In any case, the degree of replication required should be reviewed. Might it be useful for guidelines to advise on estimating power of test so as to adjust replication on a case-by-case basis?

Premature sacrifice

1. Premature sacrifice on humanitarian grounds is covered by animal testing licence regulations and must be adhered to.
2. I can see no purpose in conducting tests where birds are left to suffer or die. If the individual is clearly in distress it should be humanely destroyed immediately irrespective of the "statistical" or "scientific" requirements of the test. I can see little point in tests under such circumstances; the bird would die much earlier in the environment.
3. Perhaps dietary toxicity tests should be discontinued if birds are avoiding feeding to a point that approaches starvation. Such a test would not yield useful information on toxicity anyway.
4. Severely suffering birds should be humanely killed.
5. Euthanizing moribund animals should be allowed when it is reasonably determined that the animal will die. In addition, terminating a test or test level when it is obvious that the test substance is not being consumed and no alternate source of food is provided is appropriate.
6. If the dietary toxicity test (LC50) continues, I think that it should be modified so that animals do not have to go through the extended starvation period common in some tests with organophosphorus insecticides and probably other chemicals. The test guidelines should be modified to reduce suffering. One approach I favor is redesigning the test to focus on the lowest concentration causing mortality (whether that is defined as a LOEC or other endpoint is up for discussion).
7. Attempts should be made to develop and validate modified endpoints designed to allow the early termination of testing and reduce the duration of suffering, e.g. for moribund animals or when starvation is likely.

Doses

1. In acute toxicity testing, it should be considered to work with fixed dose level procedures as in humane tox. testing, in order to avoid excessive suffering of animals. If this were not possible, lethal effects as an endpoint should be of less priority than sub-lethal effects. An effect such as eg lethargy may in the field amount to death, therefore sub-lethal effects are just as relevant as a direct lethal effect. In this case, less animals per concentration may suffice (see proposed BBA guideline).
2. Reduction of limit doses as required in the different avian toxicity tests would improve welfare and reduce the number of birds without substantially diminishing the predictive value of the studies. In general, the present limit doses (2000 mg kg⁻¹ or 5000 ppm diet - EPA guidelines) do not reflect the possible environmental exposures arising from the label application rate of pesticides. For example, the label application rates of the non-toxic sulfonylurea - herbicides are well below 0.1 kg ha⁻¹; however, such compounds must be tested up 2000 mg kg⁻¹ for LD50 and 5000 ppm for dietary LC50 and the testing must be carried out in two avian species!
3. Testing at environmental exposure limits would reduce animal testing at the expense of durability of the endpoint. (2)
4. Tests using lower doses rising to effective ones are more humane and useful.

- In the acute oral test, it is sufficient when doses cover only the lower part of the dose-mortality curve; range-finding tests are helpful to choose doses.

Study length

- In reproduction studies, in the case of quail, the study period could easily be reduced (as proposed in the BBA guideline) as far as numbers of eggs and young are concerned. In the case of a high fertility and hatch rate, extending the study to 32 weeks is a waste of biological material and time (and naturally money!). I do not know whether all substances would show toxicity in this shorter period of e.g. 6 weeks.
- Use Japanese quail for sub-chronic and reproduction study, reduce duration of the study to 6 weeks for parent generation.
- Repro test can be shortened and provide better data with less bird stress - see Bennett, R. reports/papers.

11 Are you content with currently available guidelines for acute oral testing? (These include EPA Guideline 71-1; OECD draft guideline; FAO draft revised guideline 2.1.1)

	Yes	No
Content with current guidelines (all)	11	6
Content with EPA	1	1
Content with OECD draft	3	
Not familiar	5	

Content with current guidelines

- An area for discussion on oral tests is regurgitation.
- These protocols are designed to establish a quantitative endpoint (LD50) with confidence intervals and slope that are associated with a measured single dose. The alternative of an ALD protocol using something akin to a stair step design would not provide the slope. Together, the 3 parameters are useful in estimating risk to a population. It is important to include >1 species for testing to gain slight insight to the possible range of toxicity estimates, despite practical difficulties that might occur in testing species that can regurgitate.
- It does have shortcomings for uses beyond initial screening.
- Would like to see complete harmonisation in all studies. (2)

Not content with current guidelines

- I think it should be considered to introduce a fixed dose method in acute oral testing. If this is not possible, then I support the proposed draft OECD guideline for acute oral testing in Japanese quail. I do not think that testing for a lethal effect (in order to obtain a LD50) is very relevant.
- I feel that changes need to be made to accommodate animal use and welfare concerns as well as a redefinition of the use and meaning of the studies. The studies should emphasize flexibility, minimization of animals used, expanding the number of species tested and use of ALD techniques. Routine requirements for quail and duck data should be ended.

3. None of the guidelines specifies the types of behavioural parameters that should be noted as indicating intoxication, their subsequent use or any details of biochemical/physiological assays that should be undertaken to assess effects. Observation for any regurgitation is only mentioned in the EPA guidelines although it may have a significant effect on the ingested dose.

21 Are you content with currently available guidelines for dietary toxicity testing? (These include OECD guideline 205)

	Yes	No
Content with current guidelines (all)	6	11
Content with EPA guidelines	2	1
Content with OECD guidelines		2
Not familiar	4	

General comments

1. We do not believe that risk assessments can be based on a laboratory test of dietary toxicity. Therefore we feel the test is not fulfilling its stated purpose.
2. The OECD 205 falls between acute and chronic and is not a key endpoint for risk assessment using EPPO, EPA or E guidelines. Depending on developments towards a chronic dietary/reproduction study this study, may become even less important.
3. Not inevitably necessary for risk assessment, acute and reproduction are adequate.
4. I do not think that the current studies replicate exposure or provide useful data.
5. They may need considerable updating (2).
6. This test should not be performed as separate test, only in combination with other endpoints (e.g. reprotox.).
7. The avian dietary toxicity guidelines (OECD, OPPT, OPP) are currently being harmonised into a single guideline (850.2200) by the EPA. The differences between these three are fairly minimal, and it is expected that a satisfactory harmonised guideline will be published in the near future.

29 Are you content with currently available approaches to assessment of food avoidance? Are standard guidelines required, or can the information be obtained from food intake/body weight data from standard dietary toxicity tests? What are the advantages and disadvantages of those alternatives?

	Yes	No
Content with current guideline	2	17
Not familiar	1	
Guideline not needed	5	

Information from standard dietary toxicity tests

1. Insufficient information on palatability is obtained in the standard dietary toxicity tests to yield consistently useful data. Methods for measuring food consumption are poor in dietary studies and would have to be modified to improve reliability of data. Additionally, a 1-choice test (or no-choice as you have it in question 31) may not yield relevant data that can transfer to field settings.

2. Dietary studies give an indication of food avoidance at concentrations close to lethal threshold, which is where it is an important exposure reducing factor and particularly relevant to spray applications. I am not sure what the relevance is to full grown birds in the field eating different foods and with a complex choice.
3. Food and weight information from the LC50 tests indicates that food avoidance occurs, but not why it occurs and nothing about the concentration at which the birds begin to detect the chemical in food. Before standardizing methods for measuring food avoidance, I think we need to determine what the information means in the field and how it is going to be used in the risk assessment process.
4. Data from dietary toxicity tests are able to indicate unpalatability but the concentrations tested in these studies are usually too low compared to concentrations in treated seed. Problems exist in interpretation of food intake reduction (what means a reduction of feed intake by 70% a x ppm?) in safety assessment.
5. I believe that an adapted LC50 test can give this type of information (compound properties).
6. The approaches that are currently available vary, and it is not at all clear that specific tests would contribute much over and above the information that can be obtained from the food intake data in connection with dietary toxicity tests.
7. If time budget analysis is attached to the standard dietary toxicity tests with videoanalysis, yes the information is there without a specific test.
8. A qualitative assessment of avoidance can be made from feed consumption and body weight measurements, but more frequently quantitative data is needed. (3)
9. If food avoidance is observed then this should be the subject of further work to determine scale level and impact on finding. At present it is just reported as a straightforward finding
10. Repellency should preferably be a study seperated from the toxicology study, because it assesses exposure rather than toxicity. Repellency may occur at concentration which are non-lethal, and hence the range of concentration to be tested should not be the same. Moreover, doing a repellency study seperate from the toxicity test provides more possibilities to address concerns of animal welfare in this.
11. Responses in standard dietary tests are misleading in at least some cases. Manner of presentation and degree of nutritional stress in dietary studies are unrepresentative of conditions in the field.

Existing tests of palatability/acceptance

1. A standard guideline for dressed seed and granules would appear feasible. However, even data from standard tests would need to be supported by post registration monitoring and surveillance where significant hazard is identified.
2. Content with the German BBA approach 25-1 'Testing of baits, granules and treated seed for hazards to birds - acceptance tests'.
3. Several approaches to assessment of food avoidance exist: protocols are available from BBA and (in several draft forms) from USDA. The BBA protocol allows estimation of food avoidance for 1 day; the USDA protocol allows estimation of food avoidance for 5-days, with some guidance for multi-day field testing. Standard guidelines should be required to ensure "a level playing field". Protocols for palatability testing can be designed to give any result the scientist may choose to elicit, thus standardization is important.
4. Harmonised guidelines are urgently required, because different countries have different requirements that could be addressed in one internationally accepted test. (3) (In one case, a field study carried out by a state institute was not accepted by the regulators of that country.)

5. I think that the methods described in Kononen et al (1986) and Mason et al (1989) are adequate for an initial screening of food avoidance. I do not think that they would be adequate for use in risk assessment unless 1) they were repeated with many species of various taxons and guilds and 2) were supplemented with evidence from field studies.
6. None have been adequately validated.
7. Preference should be given to a test design that allows to judge on the safety of a compound in a certain use (e.g. seed treatment, granular formulation), taking into account the proposed application rate (e.g. BBA guidelines 25-1, 11-1, INRA - method), over a design that quantifies the reduction in feed intake in relation to the concentration in the feed.
8. Food avoidance approaches currently used are not useful. Palatability could be obtained from food intake/body weight data from an adjusted dietary toxicity test (see R. Luttik's work).
9. Currently available approaches are generally adequate, although modifications are required to explore mode of action (e.g., sensory repellency vs. food avoidance). Standard guidelines would be useful as an aid to testing by individuals not familiar with laboratory behavioural assessment methods (e.g., toxicologists, wildlife biologists).
10. Certain information may be derived from standard dietary toxicity tests; the German "BBA - Richtlinie 24 - A" is appropriate.
11. Our objection to these tests is the same as our objection to the LC50 test, the lack of demonstrated field relevance. Existing attempts to arrive at a workable test for repellency have shown the large degree to which the exact test conditions (for example the number of birds per pen, the number and proportion of treated and untreated food bowls etc.) can influence the results in a major way (see Luttik 1993 for review). This does not inspire much confidence in basing a risk assessment on any one test design.
12. The repellency properties are determined by color, shape, surface structure and other features. The palatability of granules and treated seeds can only be tested by offering this material to the birds, not by mixing the active ingredient into the standard diet. (In certain cases it might be possible to test granules and treated seeds without a.i. but including all other formulants.)
13. Most acceptance studies have been conducted with baits and treated seed. In these circumstances a single concentration only is necessary.
14. BBA is more realistic but is conducted at only one concentration - a dose-response curve is required to allow extrapolation to multiple food types, and to test consequences of residue decay. The 75:25 treated:untreated choice may not be conservative enough.

**34 Are you content with currently available guidelines for testing reproductive effects on birds?
(These include OECD guideline 206, EPA guideline)**

	Yes	No
Content with current guidelines (all)	8	16
Content draft OECD	1	
Not familiar	2	

Content with existing guidelines

1. I'm content with the German proposal for an OECD guideline 206 'Avian Sub-chronic Toxicity Test - Oral Toxicity Including Effects on Reproduction in the Japanese Quail', but not with the valid OECD guideline 206 and the EPA Guideline.
2. The avian reproductive toxicity guidelines (OECD, OPPT, OPP) are currently being harmonized into a single guideline (850.2300) by EPA. As is the situation for the avian dietary guidelines (see above), the differences between the three reproductive effects guidelines are mostly minor, and it is expected that a satisfactory harmonized guideline will be published in the near future.

Not content with existing guidelines

1. The current design can detect a limited range of problems only. The main problem may not be with the test itself as much as with the way it is used and interpreted. Our capabilities to analyze these studies has progressed since the development of these guidelines and this should be incorporated into the protocols. Guidelines should be more precise in their requirement. The current guidelines make it difficult to compare the results of different studies.
2. If the exposure period could be shortened (i.e., reduce the prebreeding exposure or duration of breeding period) without losing useful information, then test costs and time-to-completion could be reduced. This could allow the option of repeat testing if necessary.
3. These guidelines are laborious and complicated and probably do not correspond to current needs. They probably originate from the time when persistent pesticides dominated in the market, and the exposure was mainly by accumulation of relatively small doses of a.i. in animal body. Now that the birds are normally exposed for short periods to non-persistent compounds (e.g. OP's and CB's), the methods should be amended accordingly. (2) This is a more important type of lack of realism than e.g. the artificial incubation. Also, it would be desirable that the range of species used for the tests be wider, including e.g. some passerines. Further, it may be questioned whether measurement of eggshell thickness must still be considered a standard procedure
4. Considering the frequency by which such a study will be triggered, especially in the new EC annexes, the existing guidelines are much too demanding in terms of animal sacrifice, costs and time. There is a real risk of this study consuming resources for very little benefits in terms of risk assessment, which might be better directed to studies of more relevance. A reduced type of study, as proposed by Germany is to be considered as highly desirable.
5. Tests are only concerned with the production of viable eggs and young and not parental behaviour, which may be as important to the survival of the young. Tests do not allow for direct transfer of pesticide from the plumage of adults to the egg and thence to the young as may occur in spray applications. Tests also do not include susceptibility of newly hatched young to chemicals.

Appendix 1: Respondents to questionnaire (31)

Name:

Richard Balcomb
Alain Baril/Brian Collins/Pierre Mineau
Richard S Bennett
Kristin E Brugger
Peter F Chapman
Dr Stuart Dobson
Dr E Ebert
Peter Edwards
Dr Anne Fairbrother
Ed Fite
Dr Raimund Grau
Andy Hart
Mark Jaber
Dr Gerhard Joermann
Mr Esa Lehtikoinen/Kaija Kallio-Mannila/Marja Luotola
Annegaaike Leopold
Robert Luttk
J Russell Mason
F. Nicholas Mastrota
Dr Reinhart Munk
Arvo Myllymaki
Ian Pitt
Nicholas L Roberts
Kees Romijn
John Schaeffer/Don Rodier/Maurice Zeeman
Edward W. Schafer, Jr
Dr Roland Solecki
Frieda Tataruch
Helen Thompson
Dr PierGiovanni Turillazzi
Bill A Williams

Annex 4

Comparison of Existing Avian Toxicity and Avoidance Tests

This Annex includes a series of tables in which the various existing avian toxicity and avoidance tests are compared. The methods included in this comparison are the following:

Avian acute toxicity tests

- OECD - German proposal (1992). 'Draft OECD Guideline For Testing of Chemicals - Avian Acute Toxicity Test - Oral Toxicity in the Japanese Quail'
- US EPA - Office of Pesticide Programs (1982). §71-1 'Avian Single Dose LD50 Test'
- US EPA - Code of Federal Regulations (1993). §797.2175 'Avian Acute Oral Toxicity Test'

Avian dietary toxicity tests

- OECD - Test Guideline 205 (1984). 'Avian Dietary Test'
- US EPA - Office of Pesticide Programs (1982). §71-2 'Avian Dietary LC50 Test'
- US EPA - Code of Federal Regulations. § 797.2050 'Avian Dietary Toxicity Test'

Avian reproduction tests

- OECD - Test Guideline 206 (1984). 'Avian Reproduction Test'
- German proposal (1992). 'Draft OECD Guideline - Avian Sub-chronic Toxicity Test - Oral Toxicity (including effects on reproduction in the Japanese quail following a 6-week administration of the diet)'
- US EPA - Office of Pesticide Programs (1982). §71-4 'Avian Reproduction Test'
- US EPA - Code of Federal Regulations (1993). § 797.2130 'Bobwhite Reproduction Test'
- § 7972.2150 and 'Mallard Reproduction Test'

Avian avoidance tests (acceptance/palatability)

- Federal Republic of Germany (1993). 25 - 1 'Testing of Baits and Treated Seeds for Hazards to Birds - Acceptance Tests'
- INRA - France (1990). 'Methodology of Acceptance of Feed and Seeds Treated by a Repulsive Substance, by Captive Birds'
- Mason, Avery, and Otis - Denver Wildlife Reserach Center, USA (1989). 'Standard Protocol for Evaluation of Repellent Effectiveness with Birds. Cage testing only: 3 tests (i, ii, iii)'

Avian Testing - Acute

(Bq): Bobwhite quail / **(Jq)**: Japanese quail / **(Md)**: Mallard duck

Guideline Parameters	OECD - German proposal (1992) 'Draft OECD Guideline for Testing of Chemicals - Avian Acute Toxicity Test - Oral Toxicity in the Japanese Quail'	US EPA §71-1 (1982) 'Avian Single-Dose LD50 Test'	US EPA Code of Fed. Reg. (1993) § 797.2175 'Avian Acute Oral Toxicity test'
End points	NOEL, LLD, LOEL	LD50 and corresponding 95% confidence intervals, slope of the dose-response line, NOEL	LD50 , 95% confidence limits, slope of the dose-response line
Test duration	At least 14 days	At least 14 days	At least 14 to 21 days
Total no. of birds per test	24	60	60
Test animals			
Species	Japanese quail (<i>Coturnix coturnix japonica</i>)	Mallard duck (<i>Anas platyrhynchos</i>) / Bobwhite quail (<i>Colinus virginianus</i>)	Mallard duck (<i>Anas platyrhynchos</i>) / Northern Bobwhite (<i>Colinus virginianus</i>)
Age	At least 6 weeks (Young adult birds)	At least 16 weeks (young and virgin adult birds)	At least 16 weeks (young and virgin adult birds) Same age ±1 week
Other information	Healthy birds, i.e. free of any avian diseases (aspergillosis, Newcastle disease, pullorum disease), without any discernible anomalies and injuries Birds from the same population of known parentage and from the same hatch	Healthy birds, of uniform size, weight and parity Any lots of birds that suffer an abnormal weight loss or suffer more than 10% mortality during the acclimation period should not be used Birds from the same hatch Pen-reared birds History of rearing practices (photoperiod, medication, food) The species should be the same as one of the two species selected for the avian dietary LC50 test (§ 71-2)	Healthy birds with no deformation, abnormalities, sickness or injuries Birds should not be used if more than 5% of the total test population die during the acclimation period Birds of uniform weight (±10%) Minimum recommended weights: 180g (Bq), 900g (Md) Birds from the same source and breeding population (with known breeding history)

Guideline Parameters	OECD - German proposal (1992) 'Draft OECD Guideline for Testing of Chemicals - Avian Acute Toxicity Test - Oral Toxicity in the Japanese Quail'	US EPA §71-1 (1982) 'Avian Single-Dose LD50 Test'	US EPA Code of Fed. Reg. (1993) § 797.2175 'Avian Acute Oral Toxicity Test'
Housing/Feeding Conditions			
Housing conditions	Indoors In groups or individually	Indoors or outdoors Caged by treatment level group	Indoors 2 pens of 5 birds (preferably of the same sex) /dose level
Pen	Appropriate size Wire pens recommended	Large enough to avoid crowding stress resulting in aggressive and self-destructive behaviour	> 500cm ² * 24cm /bird (Bq) > 1,000cm ² * 32cm /bird (Md) Pens constructed of galvanized metal, stainless steel or perfluorocarbon plastics Wire mesh (floors and external walls) Solid sheeting (common walls and ceilings)
Temperature (°C)	20 - 25 °C	Controlled if indoors	15 - 27 °C
Rel. Humidity (%)	40 - 80	Controlled if indoors	45 - 70
Light intensity (lux)	> 8L	10L:14D (fluorescent or incandescent sources)	8L:16D (fluorescent or incandescent sources)
Feed	Ad libitum	Free access Description of any antibiotics, vitamins, or food additives added to the feed preceding or during-testing	Diets should be as free from contaminants (pesticides, heavy metals) as possible
Water	Ad libitum	Free access	Ad libitum (water bottles or automatic watering devices recommended)
Test Procedures			
Min. acclimation period prior to the test	7 days	15 days	14 days
Place of acclimation			In the actual pens used in the test
Fasting period	15 - 20 hours	At least 15 hours	At least 15 hours

Guideline Parameters	OECD - German proposal (1992) 'Draft OECD Guideline for Testing of Chemicals - Avian Acute Toxicity Test - Oral Toxicity in the Japanese Quail'	US EPA §71-1 (1982) 'Avian Single-Dose LD50 Test'	US EPA Code of Fed. Reg. (1993) § 797.2175 'Avian Acute Oral Toxicity Test'
Test Procedures (cont'd)			
Test substance	Chemical name or characterisation Other names (e.g. trade name) Batch identification Solubility in water Volatility characteristics Empirical formula Degree of purity Solubility n-octanol/water partition coefficient Biodegradability Bioaccumulation The test method is not suitable for the testing of volatile substances Substances with corrosive or highly irritating effects should be excluded	Purity ("technical grade") Accurately weighed If the chemical is first dissolved in an evaporative vehicle (e.g., acetone, methylene chloride) in preparation for use with capsules, the evaporative vehicle should be completely evaporated at room temperature before the capsules are used	Chemical name, strength, purity, composition Stability
Administration of the test substance if vehicle maximum amount of vehicle/dosage (%b.w.)	Gavage or gelatin capsule Into the bird's crop Deionized water (preferred), corn oil, olive oil, sesame oil, 0.5-1% carboxymethyl cellulose in water, gum arabic Should not be toxic, synergistic or antagonistic < 0.1-1%	Gelatin capsule Into the crop (oral intubation) or the proventriculus Distilled water (preferred), corn oil, propylene glycol, 1 % carboxymethyl cellulose, gum arabic (acacia). Should not be toxic, synergistic or antagonistic < 0.1-1%	Gavage or gelatin capsule Dosing should be done in the early morning hours Distilled or deionized water (preferred), corn oil, propylene glycol, gum acacia Materials with known toxic or emetic properties should not be used 5 ml/kg-b.w.

Guideline Parameters	OECD - German proposal (1992) 'Draft OECD Guideline for Testing of Chemicals - Avian Acute Toxicity Test - Oral Toxicity in the Japanese Quail'	US EPA §71-1 (1982) 'Avian Single-Dose LD50 Test'	US EPA Code of Fed. Reg. (1993) § 797.2175 'Avian Acute Oral Toxicity Test'
Test Procedures (cont'd)			
Number of doses	At least 3	At least 5	At least 5
Choice of doses	Results of the acute oral toxicity test in the rat / Results of a range-finding test using the quail Range of toxic effects, including mortality, should be clearly discerned.	Results from "range-finding" studies Levels spaced on a geometric or logarithmic scale Factors between dose levels should attempt to produce mortality ranging from 10 to 90% and to produce at least 3 partial kills (i.e., between 0 and 100%) surrounding the estimated LD50	Results from a range-finding test: groups of a few birds are administered 3 to 5 widely-spaced doses. A series of 2, 20, 200, and 2,000 mg/kg b.w. is suggested Levels spaced geometrically Each dose level should be at least 60% of the next higher level At least 3 levels should result in mortality between, but not including, 0 and 100% and at least one level should kill more than 50% of the birds in a group 10 (common ratio: 5m+5f)
Minimum no. of animals per dose level	3m + 3f	10	10
(Vehicle) control group	Yes	Yes	Yes
Limit test	NOEL > 2000 mg/kg b.w.	LD50 > 2000 mg/kg	LD50 > 2,000 mg/kg
Min. observation period (post-dosing)	14 days	14 days	14-21 days

Guideline Parameters	OECD - German proposal (1992) 'Draft OECD Guideline for Testing of Chemicals - Avian Acute Toxicity Test - Oral Toxicity in the Japanese Quail'	US EPA §71-1 (1982) 'Avian Single-Dose LD50 Test'	US EPA Code of Fed. Reg. (1993) § 797.2175 'Avian Acute Oral Toxicity Test'
Results			
Validity of the test (quality criteria)	The test is invalid if animals in the control group die other than from causes related to injuries	If more than 1 control bird (i.e., 10%) dies during the test period, the test may be considered invalid, depending on the circumstances and apparent cause of mortality	A test is unacceptable if more than 10% of the control birds die during the test
Parameters to be examined	Toxic symptoms, mortality (daily throughout the entire period of the test) Behaviour	Toxic signs, regurgitation Gross pathology Mortality Recovery	Mortality, regurgitation, abnormal behaviour, toxic signs (laboured respiration, leg weakness, haemorrhage, convulsions, ruffled feathers, excessive aggression, toe-picking), remission of toxic signs, and cessation of abnormal behaviours among survivors (3 times on the day of dosing and at least daily throughout the test period)
Body weight of dead animals Body weight of living animals (at the beginning; day 14 or at the end)	Body weight of dead animals Body weight of living animals (at the beginning; day 14 or at the end)	Mean body weights for each group (at initiation and termination of test) Body weight of all birds (at the beginning, on day 14 and at the end)	Individual body weights (one week prior to dosing, at the beginning of the test, and at least weekly thereafter)
Food consumption for all groups Average daily food consumption for each animal	Food consumption for all groups Average daily food consumption for each animal	Total feed consumption for each group Average daily feed consumption for each dose level	Feed consumption per pen (at least weekly)
Macroscopic/pathological examination of all animals Gross necropsy of all animals	Macroscopic/pathological examination of all animals Gross necropsy of all animals	Gross necropsy (on all dead birds and sufficient numbers of surviving birds): general inspection of digestive tract, liver, kidneys, heart, spleen Marked observation of faecal material and urine	Gross pathological examinations on at least 2 or 3 birds dying at each dose level and on all control birds that die

Avian Dietary Testing

(Bq): Bobwhite quail / **(Jq):** Japanese quail / **(Md):** Mallard duck

Guideline Parameters	OECD 205 (1984) Avian Dietary Toxicity -	US EPA (1982) §71-2 'Avian Dietary LC50 Test'	US EPA Code of Fed. Reg. (1993) § 797.2050 'Avian Dietary Toxicity Test'
End-points	LC50, 95% confidence limits, NOEC, LC100, slope of the concentration-response curve	LC50 (ppm) and corresponding 95% confidence intervals, slope of the concentration-response line	LC50, 95% confidence limits, slope of the dose-response line
Total test duration	At least 8 days	At least 8 days	At least 8 days
Total no. of birds per test	70	60	60
Test Animals			
Species	Mallard duck (<i>Anas platyrhynchos</i>), bobwhite quail (<i>Colinus virginianus</i>), pigeon (<i>Columba livia</i>), Japanese quail (<i>Coturnix coturnix japonica</i>), ring-necked pheasant (<i>Phasianus colchicus</i>), red-legged partridge (<i>Alectoris rufa</i>)	Mallard duck (<i>Anas platyrhynchos</i>) / bobwhite quail (<i>Colinus virginianus</i>)	Mallard duck (<i>Anas platyrhynchos</i>) / northern bobwhite (<i>Colinus virginianus</i>)
Age	10 - 17 days 56 - 70 days (pigeon) Same age ±1 day	10 - 14 days (Bq) 5 - 10 days (Md) Same age	10 - 17 days Same age ±1 day
Other information	Healthy birds, i.e. free of any apparent malformations, free of such avian diseases as aspergillosis, Newcastle disease, pullorum Birds from the same population of known parentage If more than 5% mortality of the population due to health or unknown causes occurs during the 72-hour period preceding testing, the entire group should be rejected	Healthy birds with no obvious abnormalities, injuries, sickness or excessive mortality (of hatchlings) Birds from the same hatch Pen-reared birds Breeding history The species should be the same as one of the two species selected for the avian dietary LC50 test (§ 71-1)	Healthy birds with no deformation, abnormalities, sickness or injuries Birds from the same source and same hatch (with known breeding histories) Birds should not be used if more than 5% of the total test population die during the 72 hours immediately preceding the exposure period

Guideline Parameters	OECD (1984) 205 - Avian Dietary Toxicity -	US EPA (1982) §71-2 'Avian Dietary LC50 Test'	US EPA Code of Fed. Reg. (1993) § 797.2050 'Avian Dietary Toxicity Test'
Housing/Feeding Conditions			
Housing conditions	Indoors In groups (5 or 10 per pen) Individually (for pigeons)	Indoors or outdoors In groups (about 10 birds per pen)	Indoors In groups (10 birds per pen)
Pen	Suitable capacity, depending on species: 300 cm ² / bird (Bq/Jq) 600 cm ² / bird (Md)	35 * 100 * 24 cm high /10 birds (Bq) 70 * 100 * 24 cm high /10 birds (Md) Wire mesh (floors and external walls) Galvanized sheeting (ceilings and common walls)	> 3,000cm ² * 24cm /10 birds (Bq) > 6,000cm ² * 24cm /10 birds (Md) Pens constructed of galvanized metal, stainless steel or perfluorocarbon plastics Wire mesh (floors and external walls) Solid sheeting (common walls and ceilings)
Temperature (°C)	Depends on species and age	35 (inside brooder) 22 - 27 (outside brooder)	35 - 22 39 (during incubation - Bq) 45 - 70
Rel. Humidity (%)	50 - 75 60 - 85 (Md)	Adequate ventilation	70 (during incubation - Bq)
Ventilation	Good ventilation	Diurnal photoperiod (preferred) 24L (acceptable)	Good ventilation 14L:10D 24L (acceptable)
Light duration (hours)	12 - 16 L	(fluorescent or incandescent sources)	(fluorescent or incandescent sources)
Feed	Ad libitum The use of prophylactic medication should be avoided Description of the basal diet (source, composition, nutrients)	Ad libitum Description of any antibiotics, vitamins, or food additives added to the feed preceding or during testing Care should be taken to see that feed spillage or air contamination by volatile chemicals from pen to pen does not take place	Antibiotics or other medication should not be used in the diet before or during the test Diets should be as free from contaminants (pesticides, heavy metals) as possible Description of the basal diet (source, vehicles, vitamins and other supplements)
Water	Ad libitum	Ad libitum	Ad libitum (water bottles or automatic watering devices recommended)

Guideline Parameters	OECD (1984) 205 - Avian Dietary Toxicity -	US EPA (1982) §71-2 'Avian Dietary LC50 Test'	US EPA Code of Fed. Reg. (1993) § 797.2050 'Avian Dietary Toxicity Test'
Test Procedures			
Min. acclimation period	7 days	As long as possible	At least 7 days
Place of acclimation	In the diet	In the diet	In the actual pens used in the test or in identical pens
Administration of the test substance if vehicle maximum amount of vehicle/ kg feed	Water, corn oil Should not interfere with the toxicity of test substance < 2 %	Distilled water (preferred), corn oil, propylene glycol, 1% carboxymethyl cellulose, gum arabic (acacia) 2 %	In the diet Distilled water (preferred), corn oil, propylene glycol, gum arabic (acacia) < 2%
No. of concentration levels	At least 5	At least 4, 5 or 6 preferred	At least 5
Choice of concentrations	Results of a range-finding test The lowest treatment level should not result in compound-related mortality or other observable toxic effects Each level should be separated by a constant factor preferably not exceeding 2.0 The concentration of the test substance should be satisfactorily maintained in the diet (it should be at least 80% of the nominal concentration) throughout the exposure period	Results from range-finding study Levels spaced geometrically over a span intended to produce mortality ranging from 10 to 90% and to produce at least 3 partial kills (i.e., between 0 and 100%) surrounding the estimated LD50	Results from a range-finding test: groups of a few birds are fed 3 to 5 widely-spaced doses. A concentration series of 5, 50, 500, and 5,000 ppm is suggested Concentrations spaced geometrically Each concentration should be at least 60% of the next higher dose At least 3 concentrations should result in mortality between, but not including, 0 and 100% and at least one concentration should kill more than 50% (including 100%) and at least one level should kill less than 50% (including 0%) of the birds in a pen
Minimum No. of animals per dietary concentration (Vehicle) control group Limit test Exposure period (treated diet) Observation period (post-exposure)(clean diet)	10 Yes (2) NOEC > 5000 mg/kg feed 5 days 3 - 18 days	10 Yes LC50 > 5000 mg/kg feed 5 days At least 3 days	10 Yes 5 days 3 - 16 days

Guideline Parameters	OECD (1984) 205 - Avian Dietary Toxicity -	US EPA (1982) §71-2 'Avian Dietary LC50 Test'	US EPA Code of Fed. Reg. (1993) § 797.2050 'Avian Dietary Toxicity Test'
<p>Results</p> <p>Validity of the test (quality criteria) Parameters to be examined</p>	<p>The mortality in the controls should not exceed 10% at the end of the test Toxic signs (e.g. convulsions, lethargy), abnormal behaviour (e.g. unusual interactions with other birds), mortality (twice on day 1, daily thereafter)</p> <p>Average body weights for live birds in each pen (on days 0, 5, 8, and end of test if extended)</p> <p>Individual body weights of all birds that die during the test</p> <p>Food consumption per pen (days 0-5, 5-8, 8-end of test if extended)</p>	<p>Control mortality must not exceed 10% during the test period Toxic signs, abnormal behaviour, mortality vomiting, reduced food consumption</p> <p>Mean body weights for each group (at initiation and termination of test)</p> <p>Individual body weights if possible (at the beginning and at the end)</p> <p>Total food consumption for each group Average food consumption per pen</p> <p>Gross necropsy (on all dead birds and sufficient numbers of survivors): inspection of the GI tract, liver, kidneys, heart and spleen; examinations of subcutaneous fat for muscle deterioration Marked observation of faecal material and urine</p>	<p>A test is unacceptable if more than 10% of the control birds die during the test Mortality, abnormal behaviour, toxic signs (labored respiration, leg weakness, haemorrhage, convulsions, ruffled feathers, excessive aggression, toe-picking), remission of toxic signs, and cessation of abnormal behaviours among survivors (3 times on the day of dosing and at least daily throughout the test period)</p> <p>Average body weights for each pen (at the beginning of the test, on day 8 and weekly thereafter if the test is extended)</p> <p>Individual weights of all birds that die during the test</p> <p>Average food consumption per pen daily or every other day (2nd highest & 2nd lowest concentrations and control) and for both the exposure period and the normal 3-day post-exposure period (other pens)</p> <p>Gross pathology examinations (not required but may provide valuable information on target site, mode of action, etc.)</p>

Avian Reproduction Tests

(Bq): Bobwhite quail / **(Jq):** Japanese quail / **(Md):** Mallard duck

Guideline Parameters	OECD (1984) 206 'Avian Reproduction Test'	OECD - German proposal (1992) 'Draft OECD Guideline - Avian Sub-chronic Toxicity Test-Oral Toxicity'	US EPA (1982) §71-4 'Avian Reproduction Test'	US EPA Code of Fed. Reg. (1993) 'Bobwhite Reproduction Test' and 'Mallard Reproduction Test'
End-points	NOEC	NOEC, toxic threshold concentration in mg/kg food		
Total test duration	At least 20 weeks	10 - 11 weeks	At least 20 weeks	At least 20 weeks
Total no. of birds per test	96 - 144 (Bq/Jq) 96 - 128 (Md)	96	108 (Bq) 105 (Md)	96 - 144 (Bq) 96 - 128 (Md)
Test Animals				
Species	bobwhite quail (<i>Colinus virginianus</i>), Japanese quail (<i>Coturnix coturnix japonica</i>), Mallard duck (<i>Anas platyrhynchos</i>)	Japanese quail (<i>Coturnix coturnix japonica</i>)	bobwhite quail (<i>Colinus virginianus</i>), Mallard duck (<i>Anas platyrhynchos</i>)	Bobwhite (<i>Colinus virginianus</i>), Mallard duck (<i>Anas platyrhynchos</i>)
Age	20 - 24 weeks (±1 week) (Bq) Proven breeders (±1/2 weeks) (Jq) 9 - 12 months (±2 weeks) (Md)	At least six weeks, after the birds have reached sexual maturity	Birds approaching their first breeding season	At least 7 months old Birds approaching their first breeding season Same age ±1 month
Other information	Birds free of disease and injury	Healthy birds, free of any avian diseases, such as aspergillosis, Newcastle disease, pullorum disease, with no discernible anomalies and injuries	Healthy birds with no sickness, injuries, excessive mortality (of hatchlings) or abnormalities	Healthy birds with no deformation, abnormalities, sickness or injuries
	Birds from the same population of known parentage	Birds from the same population of known parentage and from the same hatch	Birds from the same source Pen-reared birds History of rearing practice (lighting practices, disease record, drug and any other medication administered)	Birds from the same source and same strain (with known breeding histories: lighting practices, disease record, drug and medication administered) Pen-reared birds
	A population of birds should not be used if more than 3% of either sex die or become debilitated during the acclimation period	To avoid the pairing of siblings, it should be ensured that the strain from which the test animals originate is of sufficient size		Birds should not be used if more than 3% of either sex die during the health observation period

Guideline Parameters	OECD (1984) 206 'Avian Reproduction Test'	OECD - German proposal (1992) 'Draft OECD Guideline - Avian Sub-chronic Toxicity Test-Oral Toxicity'	US EPA (1982) §71-4 'Avian Reproduction Test'	US EPA Code of Fed. Reg. (1993) 'Bobwhite Reproduction Test' and 'Mallard Reproduction Test'
Housing/Feeding Conditions				
Housing conditions	Indoors (preferred) or outdoors	Indoors	Indoors	Indoors
→ Adult	In pairs (1m:1f), or in groups: (1m:2f) (Bq/Jq) (1m:3f) (Md)	In pairs (1m:1f)	In groups (1m:2f) (Bq) (2m:5f) (Md)	In pairs (1m:1f) or in groups (1m:2f) (Bq) In pairs (1m:1f) or in groups (1m:3f) (Md)
→ Young	In groups (by pen of origin) Together (if birds marked individually)			In groups (by pen of origin) Together (if birds marked individually)
Pen				
→ Adult	0.25 m2 / pair (Bq) 0.15 m2 / pair (Jq) 1 m2 / pair (Md)	Appropriate size 200 cm2 / bird	Adequate size	> 5,000cm2 / bird (Bq) > 10,000cm2 / bird (Md)
→ Young		50 cm2 / bird		
		Wire pens with slanting floors	Screen-bottomed pen and water bath (Md) Pens should be arranged to prevent cross-contamination Pen constructed of stainless steel, galvanized steel and materials coated with perfluorocarbon plastics	Pens constructed of galvanized metal, stainless steel or wire mesh Wire mesh (floors and external walls) Solid sheeting (common walls and ceilings)
Temperature				
→ Adult	22 ± 5°C	20 - 25 °C	21 °C	21 °C
→ Eggs/young birds	15-16 °C (Bq/Jq); 14-16 °C (Md) (storage) 37.5 °C (incubation/hatching) 35-38 °C (Bq/Jq); 32-35 °C (Md) (1 week old) 30-32 °C (Bq/Jq); 28-32 °C (Md) (2 weeks old)	16 ± 1°C (storage) 37.8 °C (incubation) 35 - 38 °C (1 week old) 30 - 35 °C (2 weeks old)	16 °C (storage) 39 °C (hatching)	16 °C (storage) 37.5 °C (incubation) 35-22 °C

Guideline Parameters	OECD (1984) 206 'Avian Reproduction Test'	OECD - German proposal (1992) 'Draft OECD Guideline - Avian Sub-chronic Toxicity Test-Oral Toxicity'	US EPA (1982) §71-4 'Avian Reproduction Test'	US EPA Code of Fed. Reg. (1993) 'Bobwhite Reproduction Test' and 'Mallard Reproduction Test'
Housing/Feeding Conditions (cont'd)				
Rel. Humidity (%) → Adult	50 - 75	40 - 80	55	55
→ Eggs/Young birds	55-75 (Bq/Jq); 60-85 (Md) (storage) 50-65 (Bq); 50-70 (Jq); 60-75 (Md)(incubation) 70-75 (Bq/Jq); 75-85 (Md) (hatching) 50-75 (Bq/Jq); 60-75 (Md)	60 - 70 (storage) 60 (incubation) 40 - 80	65 (storage) 70 (hatching)	55 - 80 (storage) 70 (incubation)
Ventilation	Good ventilation		Necessary	Good ventilation (rate: 4 changes per hour in winter; 15 changes in summer)
Light duration (hours) → Adult	7 - 8 L (8 weeks) 16 - 18 L (thereafter) Transition period at dawn and dusk	16L:8D (intensity: 65-100 lux)	7L (8 weeks) 16-17 L @ 19-20 L (thereafter) (intensity: 6 footcandles)	7-8 L (6-8 weeks) 16-17 L @ 19-20 L (thereafter) (intensity: 6 footcandles)
→ Young	Diurnal preferred (e.g. 14L:10D) Transition period at dawn and dusk	16L:8D (intensity: 65-100 lux)		
Egg turning	Storage: optional Incubation: yes Hatching: no	Storage: yes (daily) Incubation: yes (several times a day)		Storage: yes (daily) Incubation: yes (automatically or daily by hand)
Feed	Ad libitum Renewed at least weekly The use of chemicals or medication should be avoided Description of the basal diet (source, composition, nutrients)	Ad libitum	Ad libitum	Antibiotics or other medication should not be used in the diet or water of breeding birds Diets should be as free from contaminants (pesticides, heavy metals) as possible Description of the basal diet (source, ingredients, vitamins and other supplements) Ad libitum (water bottles or automatic watering devices recommended) Bacitracin or one of its forms may be added to the drinking water of young birds if necessary (Bq)
Water	Ad libitum	Ad libitum	Ad libitum	

Guideline Parameters	OECD (1984) 206 'Avian Reproduction Test'	OECD - German proposal (1992) 'Draft OECD Guideline - Avian Sub-chronic Toxicity Test-Oral Toxicity'	US EPA (1982) §71-4 'Avian Reproduction Test'	US EPA Code of Fed. Reg. (1993) 'Bobwhite Reproduction Test' and 'Mallard Reproduction Test'
Test Procedures				
Health observation period			2 - 6 weeks	At least 2 weeks
Min. acclimation period prior	At least 2 weeks	At least 7 days		At least 2 weeks (may coincide with the health observation period)
Place of acclimation				In the actual pens used in the test or in identical pens
Test substance	Chemical identification data; Water solubility; vapour pressure; structural formula; purity; chemical stability in water, light, and in diet; n-octano/water partition coefficient; biodegradability The test guideline cannot be used for highly volatile or unstable substances The test substance should possess characteristics which allow uniform mixing in the diet	Solubility in water; volatility Empirical formula; degree of purity; chemical stability in water, light and in the diet; n-octano/water partition coefficient; biodegradability; bioaccumulation; chemical name and other names Data on the acute oral toxicity for the bird species used must be available This test method is not suitable for the testing of volatile substance	The test substance material should be added to table grade corn oil or other appropriate vehicle and premixed with an aliquot of basal diet. The final diet should be uniformly mixed composition consisting of 98 to 99 parts by weight of basal diet and 1 or 2 parts by weight of corn oil.	Scientific name Chemical names, source, composition, known physical and chemical properties (e.g., solubility, volatility, degradation rate, etc.); degree of purity When a test substance is not water soluble, it may be dissolved in a reagent grade evaporative diluent (e.g. acetone, methylene chloride) and then mixed with the test diet. The solvent should be completely evaporated prior to feeding
Administration of the test substance (adult birds only)	In the diet	In the diet, or in special cases in the drinking water	In the diet	In the diet
if vehicle	Water, corn oil Should not interfere with the toxicity of test substance	Water, corn oil, sesame oil	Corn oil (preferred)	Distilled water (preferred), corn oil, propylene glycol, gum arabic (acacia)
maximum amount of vehicle/ kg feed	< 2%	< 2%	2%	< 2%
Test substance exposure period	Entire test period	6 weeks	Entire test period	Entire test period

Guideline Parameters	OECD (1984) 206 'Avian Reproduction Test'	OECD - German proposal (1992) 'Draft OECD Guideline - Avian Sub-chronic Toxicity Test-Oral Toxicity'	US EPA (1982) §71-4 'Avian Reproduction Test'	US EPA Code of Fed. Reg. (1993) 'Bobwhite Reproduction Test' and 'Mallard Reproduction Test'
Test Procedures (cont'd)				
No. of dietary concentrations	At least three	Three	At least two	Three
Choice of concentrations	Results of a dietary LC50 test (see TG 205) The highest concentrations should approximate one half of the LC10. Lower concentrations should be geometrically spaced at fractions of the highest dose (e.g. 1/6 and 1/36 of the highest dose) The test substance concentration must be satisfactorily maintained in the diet (it should be at least 80% of the nominal concentration)	Results from a range-finding test Appropriate dietary concentrations of the test substance should be chosen on the basis of toxicological data of preceding tests conducted on bird species in particular, but also from tests on rodents The highest concentration should be chosen at a level that reveals the toxic profile of the test substance, but causes no or few deaths among the animals. The intermediate concentration level should cause only minimal observable signs of toxicity, the lowest concentration level should not produce any evidence of toxicity The test substance concentration must be satisfactorily maintained in the diet (it should be at least 80% of the nominal concentration) 500 mg/kg diet 12 pairs	Concentrations for the test substance should be based on measured or calculated residues expected in the diet from the proposed use pattern(s). The concentrations should include an actual or expected field residue exposure level and a multiple level such as five. The highest non lethal level may be estimated from data developed from the avian dietary LC50 (§71-2)	The higher two treatment concentrations will be multiples (often 5x, 10x, or 20x) of the lowest treatment level. The highest treatment levels usually will be below lethal levels, unless predicted environmental exposure levels are high enough to approximate lethal concentrations
Limit test	1000 ppm			
Minimum no. of animals per dietary concentration	12 pairs or 12 groups (Bq/Jq) or 8 groups (Md)	12 pairs	At least 12 groups (Bq) At least 5 groups (Md)	12 pairs or groups (Bq) 12 pairs or 8 groups (Md)
(Vehicle) control group	Yes	Yes	Yes	Yes
Period prior to egg laying	10 - 12 weeks		At least 10 weeks	10-12 weeks
Post egg laying period	8 - 10 weeks		10 weeks	8-10 weeks
Egg collection period	Last 10 weeks (daily)	Entire test period	Approximately 10 weeks (daily)	10 weeks (daily)
Eggs reared: collected during...	The entire egg collection period	Last two weeks of exposure	The entire egg collection period	The entire egg collection period

Guideline Parameters	OECD (1984) 206 'Avian Reproduction Test'	OECD - German proposal (1992) 'Draft OECD Guideline - Avian Sub-chronic Toxicity Test-Oral Toxicity'	US EPA (1982) §71-4 'Avian Reproduction Test'	US EPA Code of Fed. Reg. (1993) 'Bobwhite Reproduction Test' and 'Mallard Reproduction Test'
Test Procedures (cont'd)				
Tests with candle	Prior to incubation → to detect cracks		Day 0 → eggshell cracks Day 11 (Bq) / Day 14 (Md) → fertility and early deaths of embryos Day 18 (Bq) / Day 21 (Md) → embryo survival	Day 0 → eggshell cracks Day 11 (Bq) / Day 14 (Md) → fertility and early deaths of embryos Day 18 (Bq) / Day 21 (Md) → embryo survival
Storage → Incubation	Weekly or every other week	Storage should not exceed 10 days	Weekly	Weekly or every other week (Bq) Bi-weekly (Md)
Incubation → Hatching	Day 21 (Bq); Day 16 (Jq); Day 23 (Md)		Day 21 (Bq); Day 23 (Md)	Day 21 (Bq); Day 23 (Md)
Expected hatching	Day 23-24 (Bq); Day 17-18 (Jq); Day 25-27 (Md)	Day 16-17	Day 23-24 (Bq); Day 25-27 (Md)	Day 24 (Bq); Day 27 (Md)
Rearing period	14 days	14 days	At least 14 days	14 days
Results				
Validity of the test (quality criteria)	The mortality in the controls should not exceed 10% at the end of the test; The average number of 14-day-old survivors per hen in the controls should be at least 12 (Bq), 24 (Jq) and 14 (Md) The average egg shell thickness for the control group should be at least 0.34mm (Md), 0.19 (Bq/Jq)	In the control group, the hatching success for the incubated eggs laid during the 5th and the 6th week of exposure should be at least 50%. The rate of viability should not drop below 80%. At least 10 breeding pairs of the control group should survive until the end of the test.		A test is unacceptable if: * Bq chick / Md Duckling productivity in control groups does not average twelve 14-day-old survivors per hen over a 10-week period * the average eggshell thickness in control groups is less than 0.19 mm (Bq) / 0.34 mm (Md) * more than 10% of the adult control birds die during the test
Treatment of data	Test groups individually compared to the control group by a statistical procedure designed in the study plan (e.g. analysis of variance)	Data treated by means of suitable statistical procedure	Experimental groups compared to controls by analysis of variance	A statistical analysis should be performed, preferably by analysis of variance or regression analysis

Guideline Parameters	OECD - (1984) 206 - Avian Reproduction Test -	OECD - German proposal (1992) 'Draft OECD Guideline - Avian Sub-chronic Toxicity Test-Oral Toxicity'	US EPA (1982) §71-4 'Avian Reproduction Test'	US EPA Code of Fed. Reg. (1993) 'Bobwhite Reproduction Test' and 'Mallard Reproduction Test'
<p>Results (cont'd) Parameters to be examined → Adult</p>	<p>Mortality and toxic signs (daily) Body weight (at beginning, prior to onset of egg laying, at the end) Food consumption (weekly or every other week) Gross pathological examination for all birds Residue analysis of selected tissues may be useful particularly for test substance with a value of log P (n-octanol/water) higher than 3.0</p>	<p>Abnormal behaviour (unusual interactions with other birds), toxic signs (tremor, lethargy), mortality (daily); Food consumption per pair (weekly); Body weight of the male and female birds (at the beginning of the acclimatisation period, at the beginning of treatment and at the end of treatment) Gross pathology for all animals: complete necropsy and a macroscopic pathological assessment Wet weight of heart, liver, spleen, testis and oviduct (without developing eggs) Histopathology on organs showing gross pathological changes</p>	<p>Abnormal behaviour, morphological and physiological responses, post mortem autopsy, food consumption (at last at biweekly intervals); estimates of average feed consumption (grams per day), palatability or repellency Body weights (at test initiation, prior to onset of laying and at termination) Mortality, morbidity Residue analysis of 2 or 3 tissues known from other studies to hold highest residues Toxic signs and remission Observation of faecal material and urine Gross pathology for birds that die during the test and for some survivors Gross necropsy (on all dead birds and sufficient numbers of survivors): inspection of the GI tract, liver, kidneys, heart, reproductive organs and spleen; examinations of subcutaneous fat and muscles for deterioration</p>	<p>Mortality, toxic signs (at least daily); Individual body weights (at the beginning of the test, at 14-day intervals until the onset of egg laying and at termination of treatment) Food consumption per pen (prior to the onset of laying and at least bi-weekly throughout the rest of the study) Repellency or food palatability Gross pathological examinations on all birds Analysis of two or more tissues (e.g. muscle, fat) for test substance residue is encouraged</p>
<p>→ Reproduction</p>	<p>Egg production and egg mass per pair and week; fertility; number of cracked eggs % viable embryos of eggs set for the entire test period, % hatching of eggs set from eggs laid in the 5th and in the 6th week of treatment</p>	<p>Egg shell thickness, cracked eggs, egg laid /bird /day and /season, fertility, live 3-week embryos, hatchability, dead embryos, 14-day old embryos</p>	<p>Eggshell thickness (once every two weeks) Eggs laid, cracked eggs, fertility, live 18-day embryos (Bq), live 21-day embryos (Md), hatchability</p>	
<p>→ Chicks</p>	<p>Survival of young</p>	<p>Aberrant behaviour, severe birth defects</p>	<p>Crippled survivors, weights of 14-day old embryos</p>	<p>% 14-day-old survivors, number of 14 old embryos</p>

**Avian Avoidance Tests
(Acceptability/Palatibility)**

Guideline Parameters	Federal Republic of Germany (1993) 25 - 1 - Testing of baits and treated seeds for hazards to birds - Acceptance Tests	INRA - France (1990) Methodology of acceptance of feed and seeds treated by a repulsive substance, by captive birds	Denver Wildlife Research Center USA (1989) Standard protocol for evaluation of repellent effectiveness with birds Cage testing only: 3 tests (i, ii, iii)
End/points	Poisoning, mortality	Consumption	Consumption
Total test duration (acclimation period not included)	15-17 days (observation period included)	At least 1 day	5 days
Total no. of birds per test	12-16 to 24-32 (quail) 10 to 20 (pheasant/pigeon)	10-12 to 20-24	40-50
Test Animals			
Species	Japanese quail, domestic pigeon, ring-necked pheasant (other possible species: sparrows and partridges)	Common partridge (<i>Perdix perdrix</i>), red-legged partridge (<i>Alectoris rufa</i>)	General use: Red-winged blackbird (<i>Agelaius phoeniceus</i>), European starling (<i>Sturnus vulgaris</i>), Canada geese (<i>Branta canadensis</i>) (or mallard (<i>Anas platyrhynchos</i>)) Specific use (e.g. fruit): House finch (<i>Carpodacus mexicanus</i>), American robin (<i>Turdus migratorius</i>)
Age	Adult animals	Not specified	Young adults (between 1 and 3 years old)
Other information	Healthy birds Pigeons and pheasants should not be used during their egg laying period		Healthy birds (gross index: body weight)
Housing/Feeding Conditions			
Housing conditions	In groups (quail) Individually (pheasant and pigeon)	Individually	Individually (i & ii) In groups (iii)
Pen	Bright, weatherproof Size: > 2m x 2m2 (pigeon/quail) > 2m x 6m2 (pheasant)	Outside; sheltered Birds keeping visual contact with one another Size: 1m x 1m x 1m	Birds visually isolated from one another Size: 2.0m x 1.5m x 1.5m (iii)

Guideline Parameters	Federal Republic of Germany (1993) 25 - 1 - Testing of baits and treated seeds for hazards to birds - Acceptance Tests	INRA - France (1990) Methodology of acceptance of feed and seeds treated by a repulsive substance, by captive birds	Denver Wildlife Research Center USA (1989) Standard protocol for evaluation of repellent effectiveness with birds Cage testing only: 3 tests (i, ii, iii)
Housing/Feeding Conditions (cont'd)			
Temperature	Not specified	Not specified	Indoors: 22-24 °C (room temperature) Outdoors: 0-32 °C (moderate weather conditions)
Light cycle/intensity	Not specified	Not specified	Same as those of the season in which the repellent will be used Test occurs during the same time period every day
Feeding conditions	Scattered on the floor (ad libitum) (Test substance and standard diet should not be scattered as a premixture as otherwise abraded test substance can contaminate the feed items) Quantity: daily feed demand, according to species/sex Feeding site: 2m x 2m2 (sandy soil, or in case of natural ground, suitable tiling)	In 2 feeding cups	In 2 cups, one on each side of the pen (i & iii) In one cup only (ii) Description of the diet, solvents, emulsifiers or other material used to dissolve the test substance
Ventilation	Good ventilation	Possible	
Test Procedures			
Acclimation period	7 days	7 days	5 days
Description of the feed	If <u>treated seeds are to be tested</u> : untreated seeds/bird feed mixture (1:1) If <u>baits or granules are to be tested</u> : bird feed mixture	- first 4 days: standard bird diet - remaining 3 days: untreated feed	<u>Each day</u> : 2-hour fast + 4-6-hour untreated feed (→ 22 hours for ii) + maintenance diet
Test substance	Characterization (trade name, batch no); Description (physical form and color); Active ingredient (a.i.); Concentration of a.i. in the test substance; Acute oral toxicity of the a.i. to at least one bird species (LD50 and/or lethal threshold, typical symptoms of poisoning)		Both technical grade (i.e. relatively pure) and formulated product (if available) should be tested

Guideline Parameters	Federal Republic of Germany (1993) 25 - 1 - Testing of baits and treated seeds for hazards to birds - Acceptance Tests	INRA - France (1990) Methodology of acceptance of feed and seeds treated by a repulsive substance, by captive birds	Denver Wildlife Research Center USA (1989) Standard protocol for evaluation of repellent effectiveness with birds Cage testing only: 3 tests (i, ii, iii)
Test Procedures (cont'd)			
Administration of the test substance	If treated seeds are to be tested: in treated seeds If baits or granules are to be tested: in the bird feed mixture Series A: Rigorous test 75% test substance + 25% standard diet Series B: Normal test 10% test substance + 90% standard diet	No-choice test: 100% treated feed Two-choice test: 50% treated feed (in one feeding cup) + 50% untreated feed (in the other cup) (the position of the treated feed is alternated from one cage to another)	In ways resembling those to be used in the field (in feed, baits or topically) Two-choice tests (i & iii) 50% treated feed (in one feeding cup) + 50% untreated feed (in the other cup) (the position of the treated feed is alternated daily) No-choice test (ii) 100% treated feed
Test progress	Series A conducted first Series B conducted only if mortalities occur	No-choice test conducted first Two-choice test conducted if birds eat and especially if mortalities occur	Test (i) conducted first (screening phase) Test (ii) conducted depends on various factors (potential cost/benefits, chemical's relative effectiveness) Test (iii) then conducted if significant reduction in consumption and preference ratio < 0.34
Fasting period	16 hours	none	2 hours
Duration of exposure period	8 hours (preferably beginning in the morning)	24 hours (can be extended for normal test)	4-6 hours (up to 22 hours for ii)
Cycle (fast + exposure)	Once (quail)	N/A	5 times in succession
Total treatment period	3 times in succession (pigeon/pheasant)	1 day	5 days
Observation Period (post-treatment)	1 day (quail) / 3 days (pigeon/pheasant)	Not specified	Not specified
Number of animals	2 groups of 6-8 birds (m+f) (quail) 1 group of 10 birds (m+f) (pigeon/pheasant)	10-12 to 20-24	Min. 10 birds (5m + 5f) per group
No. of treatment groups	1	1	(i) 3 (ii) 5 (iii) 1?
Concentrations of repellent			(on the basis of body weight) (i) e.g. 5.0, 2.5, 1.0% (weight/body weight) (ii) 5 concentrations bracketing the lowest effective concentration in (i) (iii) optimum concentration (from tests i & ii)
Control group	No	No	Yes

<p>Guideline Parameters</p>	<p>Federal Republic of Germany (1993) 25 - 1 - Testing of baits and treated seeds for hazards to birds - Acceptance Tests</p>	<p>INRA - France (1990) Methodology of acceptance of feed and seeds treated by a repulsive substance, by captive birds</p>	<p>Denver Wildlife Research Center USA (1989) Standard protocol for evaluation of repellent effectiveness with birds Cage testing only: 3 tests (i, ii, iii)</p>			
<p>Results</p> <p>Parameters to be examined</p> <table border="1"> <tr> <td data-bbox="384 1736 890 2047"> <p>General observation: - throughout during the 1st hour of each exposure period - hourly during the remaining 7 hours of each exposure period Symptoms of poisoning and unusual occurrences Mortality Body weights (at beginning of the 7-day acclimation period, the day before the (first) exposure period, at the end of the (last) exposure period, on the 7th and 14th days of the observation period) Macroscopic-pathological findings for all birds (especially contents of crop and gizzard for dead birds)</p> </td> <td data-bbox="384 1200 890 1736"> <p>Feed consumption (per bird and per group) During acclimation period (last 3 days): daily consumption @ average daily consumption of untreated feed (reference) <u>In case of rigorous test:</u> comparison between (treated) feed consumption and the average daily consumption of untreated feed In case of normal test: 1/ comparison between (treated) feed consumption during the last day of exposure and the average daily consumption of untreated feed 2/ % bait consumption/placebo consumption</p> </td> <td data-bbox="384 667 890 1200"> <p>Body weight Feed consumption (per bird in i & ii, per group in iii) Symptoms of post-ingestional malaise (if flavor avoidance) Preference ratio: consumption of treated feed/total consumption Treatment of data: Mean total consumption (ANOVAs, Tukey HSD tests) (i,ii, iii) Mean preference ratios (ANOVAs, Tukey HSD tests), median Food Avoidance Concentration FAC₅₀ (probit analysis), Effective Avoidance Index (EAI) (i & iii) (Sex is not considered as a variable in the analyses unless differences appear)</p> </td> </tr> </table>				<p>General observation: - throughout during the 1st hour of each exposure period - hourly during the remaining 7 hours of each exposure period Symptoms of poisoning and unusual occurrences Mortality Body weights (at beginning of the 7-day acclimation period, the day before the (first) exposure period, at the end of the (last) exposure period, on the 7th and 14th days of the observation period) Macroscopic-pathological findings for all birds (especially contents of crop and gizzard for dead birds)</p>	<p>Feed consumption (per bird and per group) During acclimation period (last 3 days): daily consumption @ average daily consumption of untreated feed (reference) <u>In case of rigorous test:</u> comparison between (treated) feed consumption and the average daily consumption of untreated feed In case of normal test: 1/ comparison between (treated) feed consumption during the last day of exposure and the average daily consumption of untreated feed 2/ % bait consumption/placebo consumption</p>	<p>Body weight Feed consumption (per bird in i & ii, per group in iii) Symptoms of post-ingestional malaise (if flavor avoidance) Preference ratio: consumption of treated feed/total consumption Treatment of data: Mean total consumption (ANOVAs, Tukey HSD tests) (i,ii, iii) Mean preference ratios (ANOVAs, Tukey HSD tests), median Food Avoidance Concentration FAC₅₀ (probit analysis), Effective Avoidance Index (EAI) (i & iii) (Sex is not considered as a variable in the analyses unless differences appear)</p>
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Annex 5

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Please note:

^F indicates that the entire publication is available from the OECD in a separate French translation. The other publications on this list are generally available in English only, but they often include a French summary.

The OECD Environment Monograph Series

*Since 1988, the Environment Monograph Series has made technical documents prepared by the OECD Environment Directorate available to the public. In mid 1996, this well received series was discontinued. **The Environmental Health and Safety Division now publishes its complimentary documents in six different series:***

Testing and Assessment;

Good Laboratory Practice and Compliance Monitoring;

Pesticides;

Risk Management;

Harmonization of Regulatory Oversight in Biotechnology; and

Chemical Accident Prevention, Preparation and Response.

Translations of the Series on Good Laboratory Practice and Compliance Monitoring into German, Russian, Polish, Czech, Slovak, Hebrew, Spanish and Italian exist or are planned.

*Some of the publications on this list are shown with an Environment Monograph number **and** one of the new series numbers. Either number can be used to order these documents. All the documents listed here were prepared by the Environmental Health and Safety Division. With the exception of publications on sale through the OECD Publications Service, copies of all these documents are available upon request at no charge directly from the Environmental Health and Safety Division (see page 187).*

Environment Monograph No. 14, *Final Report of the Expert Group on Model Forms of Agreement for the Exchange of Confidential Data on Chemicals (1988)^F*

No. 15, *Final Report of the Working Group on Mutual Recognition of Compliance with Good Laboratory Practice (1988)^F*

No. 17, *The Use of Industry Category Documents in Source Assessment of Chemicals (1989)^F*

No. 24, *Accidents Involving Hazardous Substances (1989)^F*

No. 25, *A Survey of Information Systems in OECD Member Countries Covering Accidents Involving Hazardous Substances (1989)^F [out of print]*

No. 26, *Report of the OECD Workshop on Ecological Effects Assessment (1989)^F*

No. 27, *Compendium of Environmental Exposure Assessment Methods for Chemicals (1989)^F*

No. 28, *Workshop on Prevention of Accidents Involving Hazardous Substances: Good Management Practice (1990)^F*

No. 29, *Workshop on the Provision of Information to the Public and on the Role of Workers in Accident Prevention and Response (1990)^F*

No. 30, *Workshop on the Role of Public Authorities in Preventing Major Accidents and in Major Accident Land-Use Planning* (1990)^F

No. 31, *Workshop on Emergency Preparedness and Response and on Research in Accident Prevention, Preparedness and Response* (1990)^F

No. 35, *A Survey of New Chemicals Notification Procedures in OECD Member Countries* (1990)^F

No. 36, *Scientific Criteria for Validation of In Vitro Toxicity Tests* (1990)^F

No. 39, *International Survey on Biotechnology Use and Regulations* (1990)^F

OCDE/GD(91)102 *Users Guide to Hazardous Substance Data Banks Available in OECD Member Countries* (1991)^F **[out of print]**

OCDE/GD(91)103 *Users Guide to Information Systems Useful to Emergency Planners and Responders Available in OECD Member Countries* (1991)^F **[out of print]**

[The two Users Guides above were translated into Spanish by UNEP IE.]

No. 43, *International Directory of Emergency Response Centres* (1992)^F
[under revision by the OECD and UNEP IE]

[The International Directory is a co-operative project of OECD and UNEP IE. The emergency response centres in the Directory are located in OECD and non-OECD countries.]

No. 44, *Workshop on Prevention of Accidents Involving Hazardous Substances: The Role of the Human Factor in Plant Operations* (1992)

No. 45, *The OECD Principles of Good Laboratory Practice [Series on Good Laboratory Practice and Compliance Monitoring No. 1]* (1992)^F

No. 46, *Guides for Compliance Monitoring Procedures for Good Laboratory Practice* (1992)^F

[**superseded** by Environment Monograph No. 110, *Revised Guides for Compliance Monitoring Procedures for Good Laboratory Practice* (1995)]

No. 47, *Guidance for the Conduct of Laboratory Inspections and Study Audits* (1992)^F

[**superseded** by Environment Monograph No. 111, *Revised Guidance for the Conduct of Laboratory Inspections and Study Audits* (1995)]

No. 48, *Quality Assurance and GLP [Series on Good Laboratory Practice and Compliance Monitoring No. 4]* (1992)^F

No. 49, *Compliance of Laboratory Suppliers with GLP Principles [Series on Good Laboratory Practice and Compliance Monitoring No. 5]* (1992)^F

No. 50, *The Application of the GLP Principles to Field Studies [Series on Good Laboratory Practice and Compliance Monitoring No. 6]* (1992)^F

No. 51, *Guiding Principles for Chemical Accident Prevention, Preparedness and Response: Guidance for Public Authorities, Industry, Labour and Others for the Establishment of Programmes and Policies related to Prevention of, Preparedness for, and Response to Accidents Involving Hazardous Substances Areas* (1992)^F

[The Guiding Principles are also available from the OECD in Russian and may be translated into other languages. In 1996, two Guidance Documents to be used in conjunction with the Guiding Principles were published (see below). For more information, please contact the Environmental Health and Safety Division.]

No. 52, *Report of the OECD Workshop on Monitoring of Organisms Introduced into the Environment* (1992)

No. 58, *Report of the OECD Workshop on Quantitative Structure Activity Relationships (QSARS) in Aquatic Effects Assessment* (1992)

No. 59, *Report of the OECD Workshop on the Extrapolation of Laboratory Aquatic Toxicity Data to the Real Environment* (1992)

No. 60, *Report of the OECD Workshop on Effects Assessment of Chemicals in Sediment* (1992)

No. 65, *Risk Reduction Monograph No. 1: Lead. Background and National Experience with Reducing Risk* [**Series on Risk Reduction No. 1**] (1993)

No. 66, *Report of the OECD Workshop on Strategies for Transporting Dangerous Goods by Road: Safety and Environmental Protection* (1993)

[The OECD's Chemical Accidents Programme and Road Transport Research Programme co-operated in organising this workshop.]

No. 67, *Application of Structure-Activity Relationships to the Estimation of Properties Important in Exposure Assessment* (1993)

No. 68, *Structure-Activity Relationships for Biodegradation* (1993)

No. 69, *Report of the OECD Workshop on the Application of Simple Models for Exposure Assessment* (1993)

No. 70, *Occupational and Consumer Exposure Assessments* (1993)

No. 73, *The Application of the GLP Principles to Short-term Studies* [**Series on Good Laboratory Practice and Compliance Monitoring No. 7**] (1993)^F

No. 74, *The Role and Responsibilities of the Study Director in GLP Studies* [**Series on Good Laboratory Practice and Compliance Monitoring No. 8**] (1993)^F

No. 76, *Guidance Document for the Development of OECD Guidelines for Testing of Chemicals* (1993; reformatted 1995) [**Series on Testing and Assessment No. 1f**]

No. 77, *Data Requirements for Pesticide Registration in OECD Member Countries: Survey Results* [**Series on Pesticides No. 1**] (1993)

No. 81, *Health Aspects of Chemical Accidents: Guidance on Chemical Accident Awareness, Preparedness and Response for Health Professionals and Emergency Responders Areas* (1994)^F

[Four international organisations collaborated in the preparation of this publication: the International Programme on Chemical Safety (IPCS), OECD, UNEP IE, and the World Health Organization – European Centre for Environment and Health (WHO-ECEH).]

No. 88, *US EPA/EC Joint Project on the Evaluation of (Quantitative) Structure Activity Relationships* (1994)

No. 90, *Ottawa '92: The OECD Workshop on Methods for Monitoring Organisms in the Environment* (1994)

No. 91, *Compendium of Methods for Monitoring Organisms in the Environment* (1994)

[Monographs No. 90 and 91 are companion documents.]

No. 92, *Guidance Document for Aquatic Effects Assessment [Series on Testing and Assessment No. 3]* (1995)

No. 93, *Report of the OECD Workshop on Chemical Safety in Port Areas* (1994)

[This Workshop was co-sponsored by OECD, the International Maritime Organization (IMO) and UNEP. Also see Monograph No. 188.]

No. 94, *Report of the OECD Special Session on Chemical Accident Prevention, Preparedness and Response at Transport Interfaces Areas* (1995)

No. 95, *Report of the OECD Workshop on Small and Medium-sized Enterprises in Relation to Chemical Accident Prevention, Preparedness and Response Areas* (1995)

No. 98, *Detailed Review Paper on Biodegradability Testing [Series on Testing and Assessment No. 2]* (1995)

No. 99, *Commercialisation of Agricultural Products Derived through Modern Biotechnology: Survey Results [Series on Harmonization of Regulatory Oversight in Biotechnology No. 1]* (1995)

No. 100, *Analysis of Information Elements Used in the Assessment of Certain Products of Modern Biotechnology [Series on Harmonization of Regulatory Oversight in Biotechnology No. 2]* (1995)

No. 101, *Risk Reduction Monograph No. 2: Methylene Chloride. Background and National Experience with Reducing Risk [Series on Risk Reduction No. 2]* (1994)

No. 102, *Risk Reduction Monograph No. 3: Selected Brominated Flame Retardants. Background and National Experience with Reducing Risk* [**Series on Risk Reduction No. 3**] (1994)

No. 103, *Risk Reduction Monograph No. 4: Mercury. Background and National Experience with Reducing Risk* [**Series on Risk Reduction No. 4**] (1994)

No. 104, *Risk Reduction Monograph No. 5: Cadmium. Background and National Experience with Reducing Risk* [**Series on Risk Reduction No. 5**] (1994)

No. 105, *Report of the OECD Workshop on Environmental Hazard/Risk Assessment* [**Series on Testing and Assessment No. 4**] (1995)

No. 106, *Data Requirements for Registration of Biopesticides in OECD Member Countries: Survey Results* [**Series on Pesticides No. 3**] (1996)

No. 107, *Report of the OECD Workshop on the Commercialisation of Agricultural Products Derived through Modern Biotechnology* [**Series on Harmonization of Regulatory Oversight in Biotechnology No. 3**] (1995)

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